Factors affecting the pharmacokinetics

and

pharmacodynamics of omeprazole in the horse

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Abstract

Equine Gastric Ulcer Syndrome (EGUS) is a common condition of the horse, affecting a broad range of horse usages and types. Recently the terminology of the syndrome has been expanded to include terms Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) to describe diseases of the squamous and glandular mucosa of the stomach, respectively.

Omeprazole, a proton pump inhibitor that blocks acid production, is considered the treatment of choice for EGUS and it has been widely used for this purpose for nearly 20 years. Yet, surprisingly, despite its widespread use little is known about the factors that affect its efficacy, such as the impact of formulation, diet and dose. Further, although it is commonly believed that the once daily administration of omeprazole results in durations of acid suppression exceeding 24 hours, there is conflict in the literature as to the validity of this belief. Recent clinical studies have demonstrated that the healing rate for EGGD is inferior to that of ESGD, raising further questions as to the efficacy of omeprazole under clinically relevant conditions. One potential reason for this observation is that the duration of intra-day acid suppression required for healing of ESGD may be less than that for EGGD. This, coupled with the possibility that once daily administration of omeprazole may not result in acid suppression for the entire 24 hour treatment interval, provides a potential explanation for the poor EGGD healing rates that have been recently reported.

The purpose of this thesis was to investigate the factors that affect the efficacy of omeprazole in the horse. The studies were conducted in four parts; firstly, the effect of formulation and diet on the pharmacokinetics of omeprazole were investigated; secondly, a model that allows continuous intra-gastric pH measurement under clinically relevant conditions was developed; thirdly, the impact of diet and dose on the pharmacokinetics and pharmacodynamics of omeprazole were investigated; and lastly, the relationships between key pharmacokinetic and pharmacodynamic variables were investigated.

The findings of the study suggested that some method of physical protection is required to protect the omeprazole from degradation in the acidic environment of the stomach and to improve bioavailability. However, significant differences were not present between formulations utilising the two most common forms of protection, namely the use of enteric coated granules in paste or buffering of the formulation. This suggests that the method of protection, buffering of the formulation or the use enteric coated granules in paste, used is less important than protection per se. The earlier studies of the thesis
suggested that an effect of feeding may be present on the pharmacokinetics of omeprazole but a statistically significant effect could not be demonstrated. Similarly, the latter studies suggested that diet may play a role in bioavailability with *ad libitum* roughage diets impairing absorption, but no statistically significant effect was present. A wide degree of inter-individual variation was present and the small numbers of animals used may have meant that the power of the studies was inadequate to document such an effect. However, an effect of diet on the pharmacodynamics of omeprazole was present with a lower magnitude and duration of acid suppression consistently observed in *ad libitum* roughage diets when compared with high grain/low fibre diets. An inconsistent effect of dose on the pharmacodynamics of omeprazole was observed with the effect the most pronounced in the high grain/low fibre diet. In contrast, no effect of dose was present in the *ad libitum* roughage diet, although the overall efficacy of both doses was poor under these conditions. Lastly, the key pharmacokinetic and pharmacodynamic parameters correlated poorly, which suggested that plasma concentrations are poorly predictive of pharmacodynamic response in the horse, although the reasons for this are unclear as in other species area under the curve is predictive of pharmacodynamic response.

The results of the present studies suggested that both diet and dose impact on the pharmacodynamics of omeprazole in the horse. The overall low efficacy of omeprazole under the *ad libitum* roughage dietary conditions was surprising but potentially significant. Firstly, it provides a potential explanation for the poor healing rates recently reported for EGGD. Secondly, it suggests that singular dosing recommendations that encompass all horse types and usages, as currently used, may not be appropriate. Instead the use of dosing recommendations that take into account the diet and management of the horse may be advantageous. Lastly, the findings of the thesis demonstrate that further studies into alternative dosing regimens, such as higher dose or twice daily administration, or the investigation of alternative therapeutic agents are required to address the need a therapeutic approach that allows for effective acid suppressive therapy in horses on high roughage diets.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer reviewed publications


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Conference abstracts/presentations

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- Sykes, B. W., Sykes, K. M. & Hallowell, G. D. Efficacy of a combination of Apolectol®, live yeast (CNCM I-1077) and magnesium hydroxide in the management of Equine Gastric Ulcer Syndrome in thoroughbred racehorses: A randomised, blinded, placebo controlled clinical trial.


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- Rethinking EGUS: Equine Glandular Gastric Ulcer Syndrome.
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European College of Equine Internal Medicine (ECEIM) annual conference, Prague, Czech Republic, 2014;


- Sykes, B. W., Hewetson, M., Hepburn, R. J., Luthersson, N. & Tamzali, Y. European College of Equine Internal Medicine Consensus Statement - Equine Gastric Ulcer Syndrome (EGUS) in Adult Horses.

American College of Veterinary Internal Medicine (ACVIM) annual conference, Indianapolis, USA, 2015;


- Sykes, B. W., McGowan, C. M. & Mills, P. C. Placement of an indwelling percutaneous gastrotomy (PEG) tube for the measurement of intra-gastric pH in two horses.

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**Publications included in this thesis**


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Table of Contents

Abstract .................................................................................................................................................. 2
Declaration by author ............................................................................................................................... 4
Publications during candidature ........................................................................................................... 5
  Peer reviewed publications .................................................................................................................... 5
  Book chapters ....................................................................................................................................... 6
  Conference abstracts/presentations ...................................................................................................... 7
Publications included in this thesis ....................................................................................................... 9
  Works submitted for publication and under review ........................................................................... 13
Contributions by others to the thesis ..................................................................................................... 15
Statement of parts of the thesis submitted to qualify for the award of another degree .............. 15
Acknowledgements ............................................................................................................................... 16
Keywords ............................................................................................................................................... 17
Australian and New Zealand Standard Research Classifications (ANZSRC) ......................... 17
Fields of Research (FoR) Classification ............................................................................................... 17
Table of Contents .................................................................................................................................. 18
List of Tables ........................................................................................................................................ 22
List of Figures ....................................................................................................................................... 23
List of abbreviations used in the thesis ................................................................................................. 28
  1.1 Introduction ................................................................................................................................... 29
  1.2 Literature review ............................................................................................................................. 30
    1.2.1 Diagnosis .................................................................................................................................... 30
    1.2.2 Prevalence .................................................................................................................................. 31
    1.2.3 Pathophysiology ....................................................................................................................... 32
    1.2.4 Risk factors ............................................................................................................................... 34
    1.2.5 Treatment ................................................................................................................................. 34
  1.3 Pharmacokinetics of Omeprazole in the Horse .............................................................................. 36
  1.4 Pharmacodynamics of Omeprazole in the Horse ........................................................................... 38
  1.5 Models of Intra-Gastric pH Measurement in the Horse ............................................................... 41
  1.6 Justification for the Current Thesis ................................................................................................. 43
Chapter 2 - Pharmacokinetics of intravenous, plain oral and enteric-coated oral
omeprazole in the horse ......................................................................................................................... 45
  2.1 Prelude .......................................................................................................................................... 45
  2.2 Abstract ......................................................................................................................................... 45
2.3 Introduction ........................................................................................................... 46

2.4 Materials and Methods .......................................................................................... 47
  2.4.1 Animals & animal ethics .................................................................................. 48
  2.4.2 Group allocation and sequencing ..................................................................... 48
  2.4.3 Sample extraction ............................................................................................ 49
  2.4.4 Determination of plasma omeprazole concentration ....................................... 49
  2.4.5 Pharmacokinetic evaluation ............................................................................. 50
  2.4.6 Data analysis .................................................................................................... 51

2.5 Results .................................................................................................................... 51

2.6 Discussion ............................................................................................................... 52

Chapter 3 - The effect of feeding on the pharmacokinetic variables of two commercially available formulations of omeprazole. ............................................................. 63

3.1 Prelude .................................................................................................................... 63

3.2 Abstract .................................................................................................................. 63

3.3 Introduction ............................................................................................................ 64

3.4 Materials and Methods ......................................................................................... 64
  3.4.1 Animals & animal ethics .................................................................................. 64
  3.4.2 Group allocation and sequencing ..................................................................... 64
  3.4.3 Sample extraction and determination of plasma omeprazole concentration ..... 65
  3.4.4 Pharmacokinetic evaluation ............................................................................. 66
  3.4.5 Data analysis .................................................................................................... 66

3.5 Results .................................................................................................................... 66

3.6 Discussion ............................................................................................................... 67

Chapter 4 - Pharmacokinetics and bioequivalence testing of five commercial formulations of omeprazole in the horse. .............................................................................. 73

4.1 Prelude .................................................................................................................... 73

4.2 Abstract .................................................................................................................. 73

4.3 Introduction ............................................................................................................ 74

4.4 Material and Methods ............................................................................................ 75
  4.4.1 Animals and animal ethics .............................................................................. 75
  4.4.2 Group allocation and sample collection .......................................................... 76
  4.4.3 Sample extraction and determination of plasma omeprazole concentration ..... 76
  4.4.4 Pharmacokinetic evaluation ............................................................................. 77
  4.4.5 Data analysis .................................................................................................... 77

4.5 Results .................................................................................................................... 78
7.4.2 Group allocation .............................................................. 119
7.4.3 Administration of medication ........................................... 119
7.4.4 Sample collection ............................................................. 119
7.4.5 Sample extraction and determination of plasma omeprazole concentration ........................................... 120
7.4.6 Pharmacokinetic evaluation ............................................... 120
7.4.7 Data analysis ................................................................. 120
7.5 Results .............................................................................. 121
7.6 Discussion ........................................................................ 124
7.7 Conclusions ...................................................................... 126

Chapter 8 – Pharmacokinetic/pharmacodynamic modelling of omeprazole in the horse. 129

8.1 Introduction ....................................................................... 129
8.2 Materials and Methods ......................................................... 129
  8.2.1 Animals, group allocations, sample collection and analysis ........................................... 129
  8.2.2 Data analysis ................................................................. 129
8.3 Results .............................................................................. 129
8.4 Discussion ........................................................................ 130

Chapter 9 – General Discussion ................................................ 143

Part 1 ....................................................................................... 144
  Study 1 .................................................................................. 144
  Study 2 .................................................................................. 145
  Study 3 .................................................................................. 146
Part 2 ....................................................................................... 147
Part 3 ....................................................................................... 148

Implications of the Present Study .................................................. 149
  Formulation ........................................................................... 149
  Dose ...................................................................................... 149
  Feeding Recommendations ...................................................... 151
  Inter-individual variability ....................................................... 153

Future directions ........................................................................ 153
  Formulation ........................................................................... 153
  Dose ...................................................................................... 154
  Genetics ................................................................................ 154
  Pharmacokinetic/pharmacodynamic modelling ........................................... 155

Bibliography ............................................................................ 156

Appendix 1 – Additional publications relevant to the thesis but not forming part of it ..... 180
List of Tables

Table 2.1 - Key pharmacokinetic parameters following administration of 0.5 mg/kg omeprazole intravenously.

Table 2.2 - Key pharmacokinetic parameters following administration of 4 mg/kg of enteric-coated omeprazole to fasted horses (ECO-Fasted), enteric-coated omeprazole to fed horses (ECO-Fed) and plain omeprazole to fasted horses (PL-Fasted).

Table 2.3 - Bioavailability of enteric-coated omeprazole in fasted (ECO-Fasted) and fed (ECO-Fed) horses and plain omeprazole in fasted (PL-Fasted) horses.

Table 3.1 – Key pharmacokinetic parameters of omeprazole following the administration of 2 g of either an enteric coated (ECO) or buffered (BUFF) formulation of omeprazole orally in the fed and fasted state to six horses.

Table 4.1 - Trade names and formulation details of the five commercial omeprazole formulations studied.

Table 5.1 – Summarised values for five 23 hour periods (8 am – 7 am the following day) for pH and the percentage of time below a pH of 4 recorded over five consecutive days in two horses under two different dietary conditions; ad libitum hay (HAY) and a high grain/low-fibre diet (HG/LF) consisting of 1% each of grain and hay divided into two meals. Measurement was performed at two points. Measurement point 1 was within 1–2 cm of ventral glandular mucosa. Measurement point 2 was 5 cm distal to measurement point 1. Data shown as median (IQR).

Table 7.1 – Mean and 95% confidence intervals for Area-Under-the-Curve (AUC\textsubscript{0–\infty}), C\textsubscript{max}, T\textsubscript{max} and half-life on days 1 and day 5 for the two doses (1 mg/kg and 4 mg/kg orally once daily) and two diets (high grain/low fiber (HG/LF) and ad libitum hay (HAY)). Groups with the same superscript letter are statistically different from each other (p<0.05).
List of Figures

Figure 2.1- Plasma omeprazole concentrations following the administration of 0.5 mg/kg intravenously to 11 horses (IV-Fasted group). Data presented as median [IQR].

Figure 2.2 - Plasma omeprazole concentrations following the administration of 4 mg/kg of enteric-coated omeprazole to 12 fasted horses (ECO-Fasted group). Data presented as median [IQR].

Figure 2.3 - Plasma omeprazole concentrations following the administration of 4 mg/kg of enteric-coated omeprazole to 11 fed horses (ECO-Fed group). Data presented as median [IQR].

Figure 2.4 - Plasma omeprazole concentrations following the administration of 4 mg/kg of plain omeprazole to 12 fasted horses (PL-Fasted group). Data presented as median [IQR].

Figure 3.1 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of an enteric coated formulation of omeprazole to six fasted horses.

Figure 3.2 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of a buffered formulation of omeprazole to six fasted horses.

Figure 3.3 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of an enteric coated formulation of omeprazole to six fed horses.

Figure 3.4 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of a buffered formulation of omeprazole to six fed horses.

Figure 4.1 - Scatter plots of $T_{\text{max}}$, $C_{\text{max}}$ and Area-Under-the-Curve ($\text{AUC}_{0-\infty}$) for the five formulations tested (GastroGard (GG), Abgard, (AG), Omoguard (OG), BOVA Omeprazole Granules (BO), Gastrozol (GZ)). * denotes significantly (P>0.05) different from the reference formulation (GastroGard).

Figure 4.2 - The ratio and back-transformed 90% confidence interval between the comparison formulations (Abgard, (AG), Omoguard (OG), BOVA Omeprazole Granules (BO), Gastrozol (GZ)) and reference formulation (GastroGard (GG)) for $T_{\text{max}}$, $C_{\text{max}}$ and Area-Under-the-Curve ($\text{AUC}_{0-\infty}$). Dotted lines represent limits of bioequivalence.
Figure 4.3 - Mean (+ SD) plasma omeprazole concentration over time for five commercial formulations ((Abgard, (AG), Omoguard (OG), BOVA Omeprazole Granules (BO), Gastrozol (GZ)) and reference formulation (GastroGard (GG)).

Figure 5.1 - A gastroscopic image taken following transcutaneous insertion of a 14 G x 5.25" catheter into the stomach. A length of high tensile (40 lb.) fishing line is then passed through the lumen of the catheter and into the stomach.

Figure 5.2 - A gastroscopic image showing the positioning of the button within the ventral glandular mucosa below the greater curvature (left of image) and looking towards the lesser curvature and pyloric antrum.

Figure 5.3 - An image showing placement of the percutaneous gastrotomy (PEG) tube (top) and button gastropexy (bottom) at between 11th and 12th ribs.

Figure 5.4 - A gastroscopic image showing final placement of the button gastropexy and percutaneous gastrotomy (PEG) tube. Prior to PEG tube insertion a 25 mm nylon washer is placed over the shaft of the PEG tube to provide additional security against the stomach wall.

Figure 5.5 - A gastroscopic image showing final placement of the pH probe with two measurement points 5 cm apart. The first measurement point is located approximately 1–2 cm from the surface of the mucosa. The second measurement point sits freely within the ingesta/fluid contents of the ventral stomach.

Figure 5.6 - An example of a trace of continuous pH measurement of the ventral stomach over a 23 hour period in a horse (horse 1 – day 1) receiving a high grain/low fibre (HG/LF) diet consisting of 1% each of grain and hay divided equally into two meals fed at 10:00 am and 6:00 pm. The top trace is from measurement point 1 and the lower trace is from measurement point 2.

Figure 5.7 - An example of a trace of continuous pH measurement of the ventral stomach over a 23 hour period in a horse (horse 1 – day 3) receiving a diet consisting of ad libitum hay. The top trace is from measurement point 1 and the lower trace is from measurement point 2.

Figure 6.1 - A gastroscopic image showing final placement of the pH probe with two measurement points 5 cm apart. The first measurement point is located approximately 10–
20 mm from the surface of the mucosa. The second measurement point sits freely within
the ingesta/fluid contents of the ventral stomach.

Figure 6.2 – The effect of dose (1 mg/kg PO once daily and 4 mg/kg PO once daily) on
mean %tpH>4 at two measurement points in six horses on either a high grain/low fibre diet
or an *ad libitum* hay only diet.

Figure 6.3 - The effect of dose (1 mg/kg PO once daily and 4 mg/kg PO once daily) on
mean median intra-day pH at two measurement points in six horses on either a high
grain/low fibre diet or an *ad libitum* hay only diet.

Figure 6.4 – Scatter plots demonstrating the relationship between daily measurements at
two different points (measurement point 1 and measurement point 2) 5 cm apart in the
ventral stomach of 6 horses.

Figure 7.1 – Scatter plots of Area-Under-the-Curve (AUC$_{0-\infty}$), C$_{\text{max}}$, T$_{\text{max}}$ and half-life on
days 1 and day 5 for the two doses (1 mg/kg and 4 mg/kg orally once daily) and two diets
(high grain/low fiber (HG/LF) and *ad libitum* hay (HAY)). Groups with the same superscript
letter are statistically different from each other (p<0.05).

Figure 8.1 - Mean intra-day pH vs. C$_{\text{max}}$ at measurement point 1. All data points from days
1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg
and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low
fibre diet) are included.

Figure 8.2 - Mean intra-day pH vs. C$_{\text{max}}$ at measurement point 2. All data points from days
1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg
and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low
fibre diet) are included.

Figure 8.3 - Median intra-day pH vs. C$_{\text{max}}$ at measurement point 1. All data points from
days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1
mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high
grain/low fibre diet) are included.

Figure 8.4 - Median intra-day pH vs. C$_{\text{max}}$ at measurement point 2. All data points from
days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1
mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.5 - %tpH>4 vs. \( C_{\text{max}} \) at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.6 - %tpH>4 vs. \( C_{\text{max}} \) at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.7 - Mean intra-day pH vs. AUC\(_{0-\infty}\) at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.8 - Mean intra-day pH vs. AUC\(_{0-\infty}\) at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.9 - Median intra-day pH vs. AUC\(_{0-\infty}\) at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.10 - Median intra-day pH vs. AUC\(_{0-\infty}\) at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

Figure 8.11 - %tpH>4 vs. AUC\(_{0-\infty}\) at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.
Figure 8.12 - %tpH>4 vs. AUC$_{0-\infty}$ at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.
### List of abbreviations used in the thesis

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<tr>
<td>BW</td>
<td>Bodyweight</td>
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<tr>
<td>$C_{\text{max}}$</td>
<td>Maximal plasma/serum concentrations</td>
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<tr>
<td>ECEIM</td>
<td>European College of Equine Internal Medicine</td>
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<tr>
<td>EGGD</td>
<td>Equine Glandular Gastric Disease</td>
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<td>ESGD</td>
<td>Equine Squamous Gastric Disease</td>
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<tr>
<td>EGUS</td>
<td>Equine Gastric Ulcer Syndrome</td>
</tr>
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<td>F%</td>
<td>Bioavailability</td>
</tr>
<tr>
<td>GERD</td>
<td>Gastroesophageal Reflux Disease</td>
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<td>IQR</td>
<td>Inter-Quartile Range</td>
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<tr>
<td>IM</td>
<td>Intramuscular</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-Inflammatory Drug</td>
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<tr>
<td>PEG</td>
<td>Percutaneous gastrotomy</td>
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<td>PO</td>
<td>Per Os</td>
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<tr>
<td>PPI</td>
<td>Proton Pump Inhibitor</td>
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<tr>
<td>PUD</td>
<td>Peptic Ulcer Disease</td>
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<tr>
<td>$T_{\text{max}}$</td>
<td>Time to maximal plasma/serum concentration ($C_{\text{max}}$)</td>
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<tr>
<td>$t_{1/2}$</td>
<td>Half-life</td>
</tr>
<tr>
<td>UHPLC-MS</td>
<td>Ultra High Performance Liquid Chromatography-Mass Spectrometry</td>
</tr>
<tr>
<td>%tpH&gt;4</td>
<td>The percentage of time that pH is greater than 4 in a 23 hour period</td>
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</tbody>
</table>
Chapter 1

1.1 Introduction

The horse’s stomach is a single chamber with two different linings. The top half of the stomach is lined by a squamous mucosa which is akin to the lining of the oesophagus while the bottom half of the stomach is lined by a glandular mucosa akin to the lining of the human stomach. The term Equine Gastric Ulcer Syndrome (EGUS) was first adopted in 1999 to describe ulcerative diseases of the stomach and its use has become widespread. However, the term is commonly misrepresented and recently an attempt has been made to clarify its meaning with an emphasis on recognition that the term EGUS refers to a syndrome, within which numerous disease entities exist [1]. To expand the terminology the terms Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) have been proposed by the European College of Equine Internal Medicine (ECEIM) Consensus Panel to describe diseases of the squamous mucosa and glandular mucosa, respectively [1].

Regardless of the lesion type omeprazole is recommended as the treatment of choice [1], consistent with the mantra in human medicine of “no acid, no ulcer” [2]. Early studies investigating the pharmacodynamics of omeprazole in the horse suggested that it has a 24 hour duration of activity following enteral administration [3,4] and its efficacy in the treatment of ESGD in a number of clinical studies [5–7] appeared to support this conclusion. However, recent studies performed by one of the authors in the lead up to this thesis demonstrated that only 25% of EGGD lesions heal with 28-35 days of omeprazole therapy at 4.0 mg/kg per os (PO) once daily in direct contrast to an ESGD lesion healing rate of 78% in the same studies [8–10].

The reasons for the lower EGGD response rate are not immediately apparent. One possibility is that the duration of intra-day acid suppression required for healing of ESGD may less than that required for healing of EGGD. A 2003 study by Merritt et al suggested that, in contrast to earlier reports, the duration of acid suppression with 4 mg/kg PO once day may be ≤ 14 hours [11]. As such, it may be that ≤ 14 hours of acid suppression is adequate for ESGD healing, while EGGD healing may require acid suppression of a longer duration. An alternative explanation is that the duration of therapy with current treatment guidelines is inadequate for glandular healing to occur. In humans, the duration of therapy for glandular ulceration is dependent on primary cause of the lesion [2]. For Helicobacter pylori associated ulcers, triple therapy combining antimicrobials and acid suppression
consistently yields first line eradication rates of greater than 80% with 7 – 14 days of therapy [2]. In contrast, Non-Steroidal Anti-Inflammatory Drug (NSAID) induced glandular ulceration requires longer treatment duration with 84% and 100% of patients healing at 8 and 12 weeks, respectively, in response to acid suppression therapy and discontinuation of NSAID therapy in one study [12]. A third potential explanation is that adjunctive therapy, such as antimicrobials, may be required. However, to date, a definitive role for bacteria in the pathogenesis of ESGD or EGGD has not been demonstrated and the current ECEIM consensus statement position is that, in line with the professions responsibilities regarding the responsible use of antimicrobials, the routine use of antimicrobials is not justified due to a lack of evidence to support either a role for bacteria in the pathogenesis of EGGD, or the efficacy of antimicrobials in the treatment of EGGD [1].

Given that acid suppression is considered a cornerstone of therapy in human medicine, regardless of the inciting cause or use of adjunctive therapy [2], and the conflict that exists in the literature as to duration and magnitude of acid suppression achieved with omeprazole in the horse at current dosing regimens; the authors considered that further investigation into the efficacy of omeprazole in the horse was warranted before longer treatment durations, or the use of adjunctive therapies such as antimicrobials, were investigated in detail. Further, it was considered that investigation into factors that may affect efficacy such as formulation, diet and dose was warranted.

1.2 Literature review

1.2.1 Diagnosis

Gastroscopy is currently considered the only reliable ant-mortem method for definitively diagnosing EGUS. There are currently no reliable haematological or biomechanical markers available to aid in diagnosis of gastric ulceration. A sucrose permeability test has shown promise for non-invasive detection of gastric ulcers [13,14], but to date, the diagnostic accuracy of the test has not been reported in clinical cases. An initial report suggested that faecal occult blood may be a useful screening test [15] but a more recent study found no association between the presence of gastric ulcers and the detection of either faecal albumin or haemoglobin [16].

Once identified on gastroscopy, assessment of the severity of lesions is most commonly achieved by assigning a grade that describes the mucosal appearance at different anatomic sites. In 1999 the Equine Gastric Ulcer Council proposed a 0-4 grading
system designed to assign severity based upon lesion depth, size and number [17] and recommended that the system should be adopted for both clinical and research use. Although a number of other grading systems have been described [5,18,19], a further study recommended that the 0-4 Equine Gastric Ulcer Council system be adopted as the standard EGUS scoring system due to its ease of use, and the repeatability and correlation of grades between examiners [20] and this recommendation has been further supported by the recent ECEIM consensus statement [1].

In contrast to ESGD there is minimal data on the validity of grading EGGD lesions. It appears that subjective visual assessment of severity and the histopathological appearance of the epithelium and mucosa correlate poorly [21] and the clinical relevance of the different manifestations of glandular disease are yet to be well evaluated. Lesions can differ in their epithelial appearance (hyperaemic, haemorrhagic, fibrinosuppurative, ulcerated) and in their mucosal contour (depressed, flat, raised) and variation in the histologic appearance of glandular lesions is reported [21]. Considering this; at present it is not recommended that a hierarchical grading system such as that used for ESGD be used for EGGD [1]. Instead, it is recommended that EGGD lesions be graded based on their anatomical location and gross appearance [1].

It is intuitive to believe that more severe lesions are more likely to result in clinically important disease and the use of hierarchical systems implies more severe disease with higher grades. However, although it has been suggested that there is a correlation between the severity of gastric ulceration and the severity of clinical signs [22,23], there is little evidence to support this notion [1]. Accordingly, prevalences are traditionally reported using a dichotomous assessment of normal or abnormal. Treatment outcomes are traditionally reported in a similar manner as healed or not, although improvement by at least 1 grade has also been used as measure of partial healing in some studies [5,6,8–10,24].

1.2.2 Prevalence

The prevalence of ESGD mirrors exercise intensity with the risk of disease increasing as the intensity of work increases. The highest prevalence has been reported in Thoroughbred racehorses, with > 70% of animals affected across a wide range of studies [19,25–27], and in competing endurance horses with 93% of animals affected [28]. Standardbred racehorses are also commonly affected (63 – 87% of animals [29,30]). However, ESGD is not solely a disease of high performance horses and it is seen across a
wide range of other horse types with 69% of horses used for a variety of purposes affected in a Danish study [31]. The ESGD prevalence has also been reported as 40-58% in show horses [32,33] and 54% in polo horses [34]. The prevalence of ESGD in horses at rest is variable, but it is typically lower and, when observed, tends to be less severe. An ESGD prevalence of 22% was recently reported in a population of feral horses [35].

The prevalence of EGGD has been less well documented until recently. Australian Thoroughbred racehorses have prevalences of between 47% [27] and 65% [8]. In endurance horses the prevalence is 16% outside of the competition period and 27-33% during the competition period [28,36]. A study in the UK found EGGD in 54% of 191 leisure horses and in 64% of 493 sport horses presenting for gastroscopic evaluation [37]. Comparably, 72% of Warmblood showjumpers [33], 69% of polo horses [34] and 57% of horses used for a variety of purposes have been reported to have EGGD in population studies [31,38]. An EGGD prevalence of 30% has been reported in a population of feral horses [35]. The majority of EGGD lesions in all of the above studies were found within the pyloric antrum.

1.2.3 Pathophysiology

Horses are constant but variable secretors of gastric acid [39] and the median pH of the ventral stomach of 3.0 over a normal 24-hour period reflects this [40]. Under normal conditions the consumption of roughage creates a basketball sized bolus of feed in the stomach that acts as a buffer to absorb gastric acidity. Further, swallowed saliva is likely to result in significant buffering at the level of the cardia. As a result the gastric pH at the level of cardia has a median value of approximately 7.0 [40]. Squamous ulceration occurs, fundamentally, as a result of increased exposure of the squamous epithelium, which has limited defence mechanisms, to highly acidic gastric contents [41,42]. Endogenous production of hydrochloric acid is likely the dominate aggressive agent, as discussed below, although duodenal bile salts may also play a role [43]. Any disruption of the normal stratification of gastric pH results in an increased risk of ESGD and damage occurs rapidly with evidence of acid injury evident within 30 minutes of exposure in vitro [44]. Exercise, which results in changes in intra-abdominal pressure and the pushing of acidic contents into the proximal stomach [45], is considered a key contributory factor in clinical disease.

The fermentation of non-structural carbohydrates consumed in the diet into short-chain fatty acids are likely also to contribute to squamous mucosal injury [46]. A consistent effect is observed with increased starch/grain intake associated with an increased risk of ESGD in
animals working at various levels of intensity in a number of studies. A marked increase in ulceration when non-exercising animals were stabled and fed grain at 1% of BW, 1 hour before hay was fed has been demonstrated [47]. Similarly, exceeding 2 g/kg BW of starch intake per day has been associated with an approximately two-fold increase in the likelihood of ESGD grade ≥ 2/5 [48]. However, the high rate of ESGD healing observed with omeprazole treatment, and in the absence of risk factor reduction [5–9,49,50], provides indirect evidence that gastric acid is the dominant erosive agent and that the role of short-chain fatty acids is likely to be less important.

The glandular mucosa differs fundamentally from the squamous mucosa in that under normal physiological conditions it is exposed to highly acidic gastric contents with the pH in the ventral portion of the stomach relatively stable at around 3 [40]. In contrast to ESGD, which occurs as a result of increased acid exposure in a region with limited defence mechanisms, EGGD is believed to result from a breakdown of the normal defence mechanisms that protect the mucosa from acidic gastric contents, although the specific mechanisms by which this occurs have yet to be elucidated.

Non-*Helicobacter pylori*, non-NSAID ulceration, otherwise known as idiopathic peptic ulcer disease, does occur in humans but until recently has been considered rare [2]. However, recent publications have suggested that between 10% and 30% of peptic ulcers in certain human populations may be idiopathic in nature [51]. Interestingly, largely mirroring our current state of knowledge in the horse, the aetiology of idiopathic ulceration in humans is unknown and treatment is empirically with proton pump inhibitors [51]. Further, the efficacy of acid suppression in the prevention of reoccurrence is questionable [52] further mimicking the current situation in the horse.

The role of bacteria in EGGD is controversial and a recent study suggested that both gastric-adapted bacteria and opportunistic pathogens may play a role in squamous ulceration [53]. Whether the situation is similar in the glandular mucosa is unknown but *Helicobacter*-like organisms have been identified in horses affected with EGGD in some studies [54,55]. However, other studies have failed to identify such organisms [21,38] and it appears, based on current knowledge, that it is unlikely that *H. pylori* is the primary causative agent of EGGD [1]. The role of secondary bacterial infection in the worsening or perpetuation of EGGD is unclear at this point in time.

Equally controversial is the role of NSAIDs in the development of EGGD. A variety of NSAIDs, namely flunixin, phenylbutazone and ketoprofen, have been shown to have
ulcerogenic potential at doses only 50% higher than typically recommended [56]. However, at clinical doses phenylbutazone and suxibuzone did not induce gastric ulceration when administered for 15 days [57] and the administration of NSAIDs was not identified as a risk factor for ESGD or EGGD in a recent study [58], nor was it identified as a risk factor for ESGD in two earlier studies [19,26].

1.2.4 Risk factors

A large number of management changes are imposed upon horses with the commencement of training, many of which have been documented to increase the risk of ESGD. These include exercise [45], high concentrate/low roughage diets [59], fasting [60], transport [61], stall confinement [60], the administration of hypertonic electrolytes [62] and intermittent access to water [48]. Further, significant associations have been shown between ESGD and individual trainers, a metropolitan yard location (horses trained in urban areas were 3.9 x more likely to have gastric ulcers), a lack of direct contact with other horses, solid barriers instead of rails, and talk rather than music radio in the barn [63]. Induction of ESGD can be rapid, occurring within 7 days in some studies [61,64] and the risk of disease increases with time in work [58].

To date, the risk factors for EGGD have been poorly described. In a study on Thoroughbred racehorses risk factors identified for EGGD were gender (colts are at reduced risk), trainer, no grass turnout, horses in direct contact with each other, horses not fed haylage, horses fed unprocessed grain, horses that were infrequently fed a complete diet, horses that underwent fast exercise on fewer days of the week and horses that went swimming [58]. In contrast to the wide range of factors identified in Thoroughbred racehorses, no effect of age, gender, use or month of presentation was found on ulcer location or type in a large study of UK leisure and sport horses [37]. Two recent studies in Warmblood showjumpers and polo horses found that horses competing at lower levels more likely to have glandular ulcerations than those competing at elite level [33,34], suggesting that glandular ulceration may negatively impact performance. Straw feeding and a lack of access to water in the paddock have been associated with an increased risk of EGUS in general [48].

1.2.5 Treatment

The mantra “no acid, no ulcer” acid suppression is considered a cornerstone of gastric ulcer management in humans [2]. Consistent with this, the recent ECEIM
consensus statement advised that acid suppression should be a cornerstone of treatment of ESGD, for which its use is well established, and EGGD, despite the failure to identify a cause (or causes) at this point in time [1]. Acid suppression does not directly contribute to healing. Instead the removal of on-going insult results in an environment conducive for healing to occur. A variety of drugs including proton pump inhibitors (PPIs), H2-receptor antagonists and antacids have been used for this purpose.

Omeprazole is the only PPI that has been studied under clinical conditions in the horse and until recently the majority of studies have reported treatment response rates for ESGD. It is traditionally believed that omeprazole irreversibly binds to, and inhibits, the H+/K+ ATPase (proton) pump that secretes HCl with new pumps needing to be made before acid production resumes [65]. However, the irreversible nature of the binding has been questioned as the rate of recovery of acid production exceeds that expected with de novo biosynthesis of new pumps [66]. Instead it is believed that a reduction in the disulphide bond between omeprazole and the proton pump may result in reversal of the inhibition [66]. Supporting this hypothesis, a study in rats documented that the half-time of ATPase restoration was only 15 hours, in contrast to 54 hours as predicted by de novo biosynthesis [67].

Other treatment modalities have been described in the horse but two studies have demonstrated omeprazole’s superior efficacy. In a study comparing the likelihood of ESGD being present only commercial omeprazole decreased the ESGD risk below that of a placebo, in contrast to buffers, sucralfate, H2-receptor antagonists and compounded omeprazole, none of which had any demonstrable benefit [68]. Similarly, omeprazole was demonstrated to be superior to ranitidine in a clinical study in Thoroughbred racehorses in Australia [50].

GastroGard® (Merial, Duluth, GA, USA), which utilises a buffer to protect the omeprazole [11], is the best studied formulation of omeprazole for the treatment of ESGD at its registered dose of 4 mg/kg PO once daily for 28 days as recommended by the 1999 EGUS Council [17] with ESGD healing rates of approximately 70 – 80% consistently reported [5–7,49,50]. Recently, the use of lower doses of an enteric coated formulation (Gastrozol®, Virbac, Milperra, NSW, Australia) has been evaluated and, under certain conditions, doses as low as 1 mg/kg PO once daily have been shown to be as efficacious as 4 mg/kg PO once daily in the treatment of ESGD [8]. Similarly, Gastrozol® at 1 mg/kg
PO once daily was equally effective as GastroGard® at 4 mg/kg PO once daily in a recent clinical trial [69].

Ranitidine and cimetidine work via competitively blocking the H₂-receptor on the parietal cell and their efficacy is dependent on maintaining plasma concentrations. Ranitidine, most commonly used at 6.6 mg/kg PO q 8 hours, has been shown to effectively suppress gastric acidity in experimental studies [39,70–72] and, although inferior at a population level [50,68], it provides an option for acid suppressor therapy where omeprazole is not available or ineffective [1]. Antacids can effectively reduce gastric acidity but their effect is short lived (≤ 2 hours) [70,73] and there are no clinical studies to support their use.

Until recently treatment recommendations for EGUS have not differentiated between ESGD and EGGD disease. However, in a series of recent studies only 25% of EGGD lesions healed with 28-35 days of omeprazole therapy at 4.0 mg/kg PO once daily in direct contrast to an ESGD lesion healing rate of 78% in the same studies [8–10]. The reasons for the poor response of EGGD to omeprazole therapy are not understood but three factors warrant consideration namely; the duration of intra-day acid suppression achieved with current dosing regimens, the duration of therapy required and the use of, or potential need for, adjunctive or alternative therapies. As discussed in the introduction; the authors believe that investigation into the duration of action, and the factors that affect it, is warranted before further investigation of adjunctive therapies or longer durations of treatment.

1.3 Pharmacokinetics of Omeprazole in the Horse

The pharmacokinetics of intravenous omeprazole have been described [74], as has intramuscular administration [75]. The pharmacokinetics following intravenous administration have been described as fitting a two-compartment model with a short β-half-life (t₁/₂) of 30 minutes [75] (compared with approximately 1 hour in humans and dogs [76]). Intramuscular administration has a bioavailability (F%) of 70-80% and a t₁/₂ of 45-60 minutes [75]. Despite its widespread use, surprisingly little is published on the pharmacokinetics of oral omeprazole and the factors that potentially influence the pharmacokinetics such as formulation, dose and diet are poorly investigated.

Of these formulation has been partly investigated. It has been previously reported that omeprazole is acid labile and that it requires some form of protection because
exposure to acid in the stomach followed by alkalinisation in the small intestine renders the drug inactive before absorption can occur [11]. This is primarily achieved by one of two mechanisms, either through the use of enteric coating, either as enteric granules that are encapsulated or suspended in a paste, or by combining buffering agents with the omeprazole in a paste formulation. Plain omeprazole is available as a compounded medication in the USA and Australia with apparently little or no buffering present [11,77].

Early studies reported the pharmacokinetics of enterally administered omeprazole but were likely limited, at least in part due the relatively poor sensitivity of the analytical methods used. One study evaluating the administration of enteric coated pellets in gelatine capsules at 0.7 mg/kg PO once daily found that, despite suppression of acid production being documented in all horses, the Area-Under-the-Curve (AUC) could only be determined for 5 out of 8 horses due to low concentrations of the drug being detected [78]. For those 5 horses a bioavailability of 12% and 14% on days 1 and 5 respectively, was reported [78]. In contrast to the effect observed in humans where bioavailability of enteric coated capsules increases by about 60% over 8 days of treatment [79], no cumulative effect of dosing was observed on either AUC or bioavailability [78]. A second, similar study evaluating 1.4 mg/kg of enteric coated pellets in gelatine capsules reported similar results with bioavailability only calculable for 5 of the 8 horses studied. In those horse the bioavailability was reported as 6% on day 1 and 13% on day 5 [4] suggesting that a cumulative effect of multiple dosing may be present. However, no consistent effect on AUC was observed and caution should be drawn in drawing any conclusions on an incomplete data set.

The pharmacokinetics of GastroGard®, the predominant formulation used in the horse globally, have been poorly described. A single study [3] reported its pharmacokinetics with a time to maximal serum/plasma concentration (T_max) following oral administration of approximately 1.5 hours. Until recently GastroGard® was protected by a global patent that was recognised in the majority of developed countries with the notable exception of Australia and New Zealand. This may in part explain why no direct comparisons of the difference formulations were published in the peer reviewed literature until recently. Similarly, the high efficacy of GastroGard® in the treatment of ESGD likely reduced interest in investigation into factors that affect its efficacy. Recent reports of its relative inefficacy in the treatment of EGGD [8–10] and the recent expiration of the global patent have resulted in an increased level of interest in factors that affect its, and omeprazole in general’s, efficacy. To this effect a recent publication reported the
comparative pharmacokinetics of GastroGard® and Gastrozol® [69]. The pharmacokinetics of GastroGard® at 4 mg/kg PO once daily were compared with those of Gastrozol® at 1 mg/kg PO once daily alongside the outcome of clinical EGUS cases. No difference in endoscopic outcome was reported although it warrants note than in general only mild lesions were present. The bioavailability, reported as AUC, for Gastrozol® was 1.26 times higher than for GastroGard® although not statistically significant effect was present due to the wide 95% confidence interval (0.56-2.81) [69]. However, interpretation of the results is difficult as the dose of omeprazole used for GastroGard® of 4 mg/kg PO once daily differed from the dose of 1 mg/kg PO once daily used for Gastrozol®. Yet, to the authors’ knowledge, direct comparisons of dose and as such dose linear pharmacokinetics have not been demonstrated for omeprazole in the horse. As such it cannot be assumed that the pharmacokinetics of the two doses are equivalent, and the direct comparison of different doses is not appropriate.

Likewise the potential impact of feeding on reducing bioavailability has been sparsely reported. A single study reported the effect of feeding on a GastroGard® wherein the mean (± standard error) AUC for omeprazole (as reported in ng h/mL) for fed horses was 633 (± 120) and 678 (± 150) on days 1 and 14, respectively, in fasted horses compared with 1808 (± 198) in fasted horses on day 5 [3]. This suggested that feeding may reduce the average bioavailability to as little as 1/3 of fasted values. Brief mention of the finding was made in the study’s discussion yet little emphasis has been placed in diet subsequently and the dosing recommendations for commercial formulations do not distinguish between administrations to fed or fasted animals. This is surprising given that the diet of different horse usage types varies greatly, from the roughage based diet of pleasure horses to the high grain/low fibre diet of high performance horses such as racehorses [80]. Similarly, feeding has been shown to reduce absorption of omeprazole in humans and human patients are advised to take omeprazole prior to eating [81,82].

1.4 Pharmacodynamics of Omeprazole in the Horse

The earliest reported studies on oral omeprazole in the horse reported the use of enteric-coated pellets in gelatine capsules administered via naso-gastric tube. In one study 0.7 mg/kg of enteric coated pellets was administered once daily via nasogastric tube for 5 days [78]. Samples of gastric fluid were collected via an indwelling gastric cannula to measure gastric acidity and acid output on days 1 and 5. Samples were collected in the period of 5-6 hours post administration and the horses were then infused with pentagastrin
for hours 6-8 to induce gastric acid output. Samples were collected for a second time period at 7-8 hours post administration (1 hour after the commencement of the pentagastrin infusion). Minimal effect of treatment was observed on day 1 but by day 5 all four parameters of acid production assessed (basal and pentagastrin stimulated acid output and acidity) were reduced by approximately 70%. Basal gastric fluid pH was unchanged in the treated horses but pentagastrin stimulated values rose from $1.7 \pm 0.7$ on the control day to $4.6 \pm 2.1$ on day 5 [78]. The improved efficacy on day 5 is consistent with reports from humans where day 5 levels of acid suppression following daily administration are greater than those observed on day 1 [83].

A second study reported the administration of 1.4 mg/kg of enteric coated pellets in gelatine capsules for 6 days [4]. Similar to the study described above, gastric acid output and acidity were determined at basal levels between 5-6 hours post administration followed by a 2 hour pentagastrin infusion and repeated measurements 7-8 hours post administration on days 1 and 5. On day 7, a third gastric secretion test with pentagastrin stimulation was performed between hours 24–27 after administration of the 6th dose. Similar results to the above study were reported for days 1 and 5 with the levels of suppression achieved on day 5 approximately 80% of control values in pentagastrin stimulated horses while basal acidity and acid output was decreased by 70% at 5-6 hours on day 5 in treated horses. Basal pH increased to $6.2 \pm 2.0$ on day 5 at 5-6 hours compared with $3.2 \pm 2.0$ on the control day and pentagastrin stimulated pH at 7-8 hours on day 5 was $4.6 \pm 2.2$ compared with control values of $1.7 \pm 0.7$ [4]. Together these findings suggested that a dose response was present with the higher dose used in this study (1.4 mg/kg PO once daily) more efficacious than the dose reported in the earlier study (0.7 mg/kg PO once daily) [78]. On day 7 basal acid output at 24-25 hours was decreased by 90% while acidity was decreased by 66%. Mean basal pH ($5.6 \pm 1.7$) appeared higher than control values ($3.2 \pm 2.0$) but was not statistically significantly different. Pentagastrin stimulated acid output at 26-27 hours was reduced by 72% and mean pH ($3.2 \pm 1.5$) was significantly higher than control values ($1.7 \pm 0.7$) [4]. These findings suggested that at this dose the duration of activity of omeprazole may be at least 27 hours in the horse under the conditions studied.

Omeprazole pharmacodynamics were further reported in a study where omeprazole was administered via nasogastric tube as acid stable granules in a methylcellulose paste at 1.5 and 5.0 mg/kg once daily, or as an oral paste of acid stable granules at 1.5 mg/kg, or an oral paste of omeprazole powder at 3.0 mg/kg PO once daily [84]. Basal gastric acid
output and pH were determined 2-3 hours post administration followed by a 3 hour pentagastrin infusion (hours 3-5) during which stimulated values were recorded. A gastric cannula model was used and horses were fasted 18-24 hours prior to each study. A clear dose effect was present between the two doses of acid stable granules studied with 5.0 mg/kg PO once daily resulting in 97% and 98% suppression of basal and pentagastrin stimulated output respectively on day 5. In comparison basal and pentagastrin stimulated output were reduced by only 53% and 57% respectively in the 1.5 mg/kg PO once daily group [84]. Similarly, mean gastric pH was 7.2 (basal) and 6.8 (pentagastrin stimulated) in the 5.0 mg/kg groups, compared with 2.6 (basal) and 2.2 (pentagastrin stimulated) in the 1.5 mg/kg group. No differences were present between days 5, 12 and 19 of treatment.

Administration of plain omeprazole powder as a paste at 1.5 mg/kg produced similar results to those reported for the nasogastrically administered acid stable granules above [84]. A dose effect was present as when the daily dose of plain omeprazole powder was increased to 3.0 mg/kg it resulted in a reduction of gastric acid output by 82% (basal) and 77% (pentagastrin stimulated) from baseline. Likewise, in the 3.0 mg/kg group mean pH was 5.2 (basal) and 4.7 (pentagastrin stimulated) compared with 3.4 (basal) and 3.0 (pentagastrin stimulated) in the 1.5 mg/kg group [84]. The authors concluded that the results achieved with the use of acid stable granules in paste form were comparable to those achieved using nasogastric intubation as the means of administration and that the use of paste formulations of omeprazole for inducing acid suppression was clinically feasible.

The same year another study reported the efficacy of an omeprazole paste at 4 mg/kg and 5 mg/kg PO once daily [3]. Basal and pentagastrin stimulated gastric acid output and pH was evaluated 5-8 hours after 5 doses, 13-16 hours after 10 doses and 21-24 hours after 15 doses. Similar to the above studies horses were fasted for 18 – 24 hours prior to the initiation of each collection. The results of the study suggest that at 4 mg/kg PO once daily profound acid suppression was achieved with both basal and pentagastrin stimulated gastric acid output reduced by 99% on day 5 (hours 5-8) and by 83% (basal) and 90% (pentagastrin stimulated) on day 15 (hours 21-24) [3]. Similarly gastric pH was 7.4 ± 0.07 and 7.03 ± 0.22 (basal and pentagastrin stimulated, respectively) at 6-8 hours on day 5, 6.51 ± 0.27 and 5.87 ± 0.44 (basal and pentagastrin stimulated, respectively) at 14-16 hours on day 10 and 5.5 ± 0.47 and 4.32 ± 0.62 (basal and pentagastrin stimulated, respectively) at 22-24 on day 15. No differences were present between the 4.0 mg/kg and 5.0 mg/kg dose [3]. The findings of this study were considered
good evidence of omeprazole’s twenty-four hour duration of activity following oral administration in the horse.

This statement is challenged by a more recent study performed by Merritt et al using pH probes inserted retrograde into the indwelling gastric cannula [11]. In this study GastroGard® was compared to three compounded formulations of omeprazole with intra-gastric pH of the ventral stomach continuously recorded for 24 hour periods in fed horses. In this study the percentage of time above a pH of 4 was used as the determinant of efficacy as this is widely accepted in human medicine as the primary determinant of symptomatic control and healing for the treatment of gastroesophageal reflux disease (GERD), a condition the has many similarities with ESGD [11]. The findings of the study were striking in that the mean hourly % time above pH 4 for GastroGard® was only 14 and 11 hours on days 2 and 7 of treatment, respectively.

If the effect described by Merritt et al [11] is repeatable then it provides a potentially simple explanation for the discrepancy in the healing rates of ESGD and EGGD as discussed in the preceding section. Simply, current dosing regimens of omeprazole may not result in an adequate duration of acid suppression, particularly in the ventral stomach, to allow EGGD lesions to heal.

1.5 Models of Intra-Gastric pH Measurement in the Horse

A variety of models have been used to measure intra-gastric pH in the horse and given the significance placed on the studies’ findings, the authors believe that discussion of the different models is warranted. A review on the history of intra-gastric measurement in the horse has recently been published [85] with the earliest report dating back to 1933 ([86] cited in [85]) when a gastric fistula was created in a horse allowing collection of gastric fluid and quantification of daily output from the live animal.

Early work in the 1990s evaluating the efficacy of different formulations of omeprazole in the horse was primarily performed using a gastric cannulation model [3,4,75,78,84] which was considered the model of choice at the time [85]. An advantage of this method was that it allows the measurement of gastric fluid pH, electrolytes and prostaglandins and the calculation of total daily gastric acid output. However, a key disadvantage was that it may not be truly reflective of clinical conditions since the horses in the studies cited above were fasted for 18 – 24 hours prior to the collection of gastric fluid. Three factors associated with fasting potentially influence the interpretation of the
studies’ results. Firstly, it is not apparent from the publications if the horse were fasted at the time of administration of the omeprazole. This is a potentially relevant as the bioavailability of omeprazole may be affected with serum levels of omeprazole, as measured by AUC, reported from fed horses being as little as 1/3 of levels obtained from fasted horses [3] depending on the formulation studied. Additionally, it is well recognised that omeprazole is most effective on parietal cells that are being stimulated [87] and the act of fasting either prior to, or following, omeprazole administration, potentially influences the interpretation of the efficacy of omeprazole. In the above studies, pentagastrin stimulation was used in an attempt to address this, but it is not known if the pentagastrin stimulation model used reliably replicates the effect of feeding induced parietal cell stimulation. Lastly, it has been suggested that fluid collected from fasted horses is likely to be influenced by contributions from both saliva and duodenal reflux [11], both of which are highly alkaline, which may confound interpretation of fluid retrieved.

A modification of the gastric cannulation model wherein a pH probe is fitted retrograde into the gastric cannula has been described [11]. This model allows the measurement of intra-gastric pH regardless of dietary management and as such it obviates some of the concerns raised with the fluid collection in the model described above. Two key disadvantages of the model are that placement of the gastric cannulas requires general anaesthesia and surgical expertise and that total daily gastric acid output cannot be determined. Although it has been argued that total daily gastric acid output may be a more sensitive marker of the effect of acid suppressive drugs [85], the clinical relevance of the it is not clear as intra-gastric pH, and specifically the percentage of time that pH exceeds 3 or 4 within each inter-treatment period are considered the primary determinants of efficacy for glandular and squamous disease, respectively, in humans [88].

As an alternative to the invasive, irreversible gastric cannulation models the placement of indwelling pH electrodes through the biopsy channel of an endoscope or nasogastric tube has been described [39,40,71,72,89]. Advantages of this model include its ease of use and the ability to study horses while they are fed or fasted. However, a possible disadvantage of this model is that the presence of the tube in the oesophagus, pharynx and nostrils may affect eating behaviour, although anecdotally this does not appear to be a significant factor. The use of mercury-weighted balloons to seat the probe ventrally has been described [70,71], but the exact location of the pH probe is unknown.
and the use of mercury presents significant occupational health and safety concerns. Due to the limitations of this technique its use has fallen out of favour.

A final method for evaluating intra-gastric pH by aspirating gastric fluid during endoscopic examination has also been described \cite{85,90–92}. The advantages of this technique are that it is simple, quick and easy to perform. However, the technique requires fasting of the horse to collect a representative sample of fluid and is therefore potentially influenced by the same factors that influence the gastric cannulation fluid collection model, namely the potential for fasting to impact on drug bioavailability, the effect of the loss of feeding induced parietal cell stimulation and the potential for mixing of saliva and duodenal reflux to confound the results. Further, the technique does not lend itself to the sequential measurement of intra-gastric pH over consecutive days.

1.6 Justification for the Current Thesis

As discussed above, despite its widespread use for over 20 years, the pharmacodynamics of omeprazole under clinically relevant conditions are poorly understood. Further, the factors that affect pharmacodynamics and pharmacokinetics of omeprazole in the horse are poorly elucidated. The recent recognition that EGGD lesions fail to heal at a rate comparable with ESGD lesions \cite{8–10} has reinvigorated interest in the overall efficacy of omeprazole as a suppressor of gastric acidity in the horse. Specifically, conflicting evidence in the current literature raises the question as to whether levels of acid suppression that are likely to result in EGGD healing are achieved under clinically relevant conditions. Further, the expiration of the GastroGard® patent has resulted in an increased in the role of formulation as a number of generic formulations have been released onto the market. Lastly, EGUS, and in particular EGGD, is becoming increasingly recognized in non-racehorse populations \cite{31}, many of which consume high roughage based diets, and the role of diet in the efficacy of omeprazole is becoming increasingly recognized as a potentially important factor.

Given the potential impact of fasting to inadvertently increased the efficacy of the drug in the cannula/pentagastrin stimulation model used in many of the early studies investigating the efficacy of omeprazole in the horse, compared with the more clinically relevant conditions of the Merritt et al \cite{11} study in 2003, the authors considered that re-evaluation of the efficacy of omeprazole using a clinically relevant model was justified. This formed the cornerstone of the justification for the current thesis. Further, given that the factors that potentially affect the efficacy of orally administered omeprazole including
formulation, dose and diet are poorly described a further justification for the thesis is to fill holes in the existing knowledge base related to these. The aims of the current thesis were to investigate;

- The role of formulation, diet and dose on the pharmacokinetics of omeprazole in the horse;

- The role of diet and dose on the pharmacodynamics of omeprazole in the horse under clinically relevant conditions;

- The feasibility of pharmacokinetic/pharmacodynamic modelling of omeprazole in the horse.
Chapter 2 - Pharmacokinetics of intravenous, plain oral and enteric-coated oral omeprazole in the horse.

This chapter consists of a paper published as:


2.1 Prelude

It is generally recommended that omeprazole requires some form of protection because exposure to acid in the stomach followed by alkalinisation in the small intestine renders the drug inactive before absorption can occur [11]. However, the magnitudes of effect of different mechanisms of protection have been poorly described. A bioavailability of 6 - 12% on day 1 of administration of enteric coated granules in a gelatine capsule has been previously reported, although blood levels of omeprazole were undetectable in 3/8 horses in each of the studies [4,78] limiting the interpretation of such studies. One of the reasons for the inability to detect blood levels of omeprazole despite an apparent acid suppressive effect that was observed concurrently is the relatively poor sensitivity of the analytical methods used in the earlier studies compared to modern analytical techniques.

The objectives of this study were to;

- Document the pharmacokinetics of intravenous, enteric-coated oral and plain (unprotected) oral omeprazole in fasted horses;
- Investigate the impact of feeding on the bioavailability of an enteric-coated formulation of omeprazole.

2.2 Abstract

The objectives were to document the pharmacokinetics of intravenous, enteric-coated oral and plain oral omeprazole in fasted horses, and to investigate the impact of feeding on the bioavailability of an enteric-coated omeprazole. Twelve horses received four treatments: Intravenous (IV) omeprazole (0.5 mg/kg) in the fasted state (IV-Fasted), enteric-coated omeprazole (4 mg/kg) orally in the fasted state (ECO-Fasted), enteric-coated omeprazole (4 mg/kg) orally in the fed state (ECO-Fed) and plain omeprazole (4 mg/kg) orally in the fasted state (PL-Fasted). Plasma omeprazole concentrations were
determined by Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS). Bioavailability was higher (p=0.038) in the ECO-Fasted group (21.5 [9.0-27.7] %) than the PL-Fasted group (10.1 [7.7-13.3] %). Similarly AUC$_{0-\infty}$ was higher in the ECO-Fasted group than the PL-Fasted group (p=0.027). No significant differences were present between the ECO-Fasted and ECO-Fed groups with regards to bioavailability, maximum plasma concentration (C$_{\text{max}}$), T$_{\text{max}}$ or AUC$_{0-\infty}$. When the t$_{1/2}$ data from the oral formulations was pooled it was longer than that observed in the IV-Fasted group (100 [73-118] min) and 35 [34-39] min, respectively; p<0.0001). The bioavailability of enteric-coated omeprazole was higher than previously reported and feeding had minimal impact. Bioavailability of plain omeprazole was approximately half that of enteric-coated omeprazole. The longer t$_{1/2}$ observed following oral administration was consistent with the flip-flop effect and has not previously been described for omeprazole in the horse.

2.3 Introduction

Equine gastric ulcer syndrome (EGUS) remains a common condition of performance horses with omeprazole considered a cornerstone of therapy [93]. GastroGard®, which utilises a highly alkaline medium to buffer and protect the omeprazole from degradation by gastric acidity [11], is the most thoroughly evaluated formulation and numerous studies have documented its efficacy at a dose of 4 mg/kg PO once daily in the treatment of EGUS [5–7,50,94,95]. Recently, enteric-coated omeprazole at a dose of 1 mg/kg PO once daily has been demonstrated to be non-inferior to enteric-coated omeprazole administered at the reference dose (4 mg/kg PO once daily) [8] in the treatment of naturally occurring gastric ulceration in clinical patients. This is in direct contrast to a similar study in which a lower dose (1.6 mg/kg PO once daily) of buffered omeprazole was inferior to the reference (4 mg/kg PO once daily) dose [10], suggesting that the bioavailability of enteric-coated formulations may be higher than buffered formulations, thereby allowing the use of lower doses with similar efficacy.

An early study evaluating an enteric-coated formulation of omeprazole administered orally reported minimal pharmacokinetic data with undetectable serum concentrations in 3/8 horses studied [4]. Utilising data from the five horses for which serum omeprazole concentrations were detectable, an oral bioavailability of 6 - 13 % was reported, with variable absorption resulting in omeprazole plasma AUC values varying by over 300% [4]. Another study reported undetectable serum concentrations in 3/8 horses, a similar wide variation in AUC [78] and a bioavailability of 12 – 14% in the remaining animals [78].
Adequate acid suppression, as measured by the collection of gastric fluid through an indwelling percutaneous cannula in fasted horses, was observed in all horses [4,78]. The reasons for the failure to detect serum concentrations of omeprazole in some animals were not explained.

The impact of feeding on the bioavailability of omeprazole in the horse is also poorly described. One study evaluating a buffered formulation of omeprazole reported that fasted horses had a 300% greater AUC compared to fed horses administered the same dose [3]. Given that AUC is the primary determinant of the magnitude and duration of acid suppression achieved following the administration of omeprazole in other species [66], this finding is potentially relevant in the clinical setting; yet no follow up studies have been performed to document the effect of feeding on the bioavailability. Further, to the authors’ knowledge, the effect of feeding on the bioavailability of enteric-coated formulations has not been reported.

It has been previously reported that omeprazole requires some form of protection because exposure to acid in the stomach followed by alkalinisation in the small intestine renders the drug inactive before absorption can occur [11]. However, a generic formulation of plain, unprotected omeprazole has recently been released onto the European market (Peptizole®, Norbrook, Co. Down, UK). The use of an unprotected formulation conflicts with previous recommendations [11], but evaluation of the potential efficacy of this formulation is difficult as little published information on bioavailability of plain omeprazole is available.

Considering this; the objectives of this study were: 1) to document the pharmacokinetics of intravenous, enteric-coated oral and plain (unprotected) oral omeprazole in fasted horses; and 2) to investigate the impact of feeding on the bioavailability of an enteric-coated formulation of omeprazole.

2.4 Materials and Methods

The omeprazole for intravenous injection was sourced from a human compounding pharmacy (Think Pharmacy, Aspley, QLD, Australia) as a powdered USP grade omeprazole (99.8% purity). It was stored at 4°C, as per the manufacturer’s instructions, until reconstituted immediately prior to administration. The plain omeprazole for oral administration was sourced from a commercial veterinary compounding pharmacy (BOVA Compounding, Sydney, NSW, Australia) as a paste formulation. It consisted of uncoated
USP grade omeprazole (99.8% purity) formulated into a paste within an almond oil suspension at a concentration of 100 mg/mL. Stability of the formulation was reported by the compounder as 3 months. The enteric coated formulation (Gastrozol®) studied was a commercially available, 50 mg/mL paste formulation for oral administration.

2.4.1 Animals & animal ethics

Twelve healthy, adult, privately owned Thoroughbred horses (aged 5 – 15 years, 406 – 606 kg BW) consisting of six male castrates and six females were used. During the studies horses were housed in a 12 m² stable bedded with wood shavings and fed as per the protocol for the allocated treatment (see below). A washout period of at least 1 week separated the treatments during which time horses were allowed access to pasture supplemented by good quality alfalfa hay.

The study was performed under an ethics permit from the New South Wales Department of Primary Industries (TRIM 12/4903). Informed consent from the owner, or the trainer acting as an agent for the owner, was obtained at the time of enrolment to the study.

2.4.2 Group allocation and sequencing

Each horse received four treatments: Intravenous omeprazole (0.5 mg/kg) in the fasted state (IV-Fasted), enteric-coated omeprazole (4 mg/kg) orally in the fasted state (ECO-Fasted), enteric-coated omeprazole (4 mg/kg) orally in the fed state (ECO-Fed) and plain (unprotected) omeprazole (4 mg/kg) orally in the fasted state (PL-Fasted). Horses were blocked into groups of four animals with the horses within each block receiving all four treatments before being replaced by the subsequent block of four horses in an all-in/all-out manner. To accommodate the effect of time, the four horses within each block were randomized on week 1 into one of the four treatment groups and subsequently managed in a cross-over design such that each treatment was administered to a single horse each week.

The omeprazole for intravenous use was reconstituted with 2.25 mmol/L NaOH in sterile water to a concentration of 5 mg/mL. The solution was administered through a 0.2 μm filter (Minisart®, Sartorius, Dandenong Sth, VIC, Australia) within 15 min of reconstitution, and over a period of 5 min, as previously described [74].
To ensure accurate dosing each horse was weighed on electronic scales (Accuweigh Equestrian Scales®, Accuweigh, Willeton, WA, Australia) the day prior to each investigation. For three of the investigations (IV-Fasted, ECO-Fasted, PL-Fasted) the horses were fasted for 16 hours to ensure adequate emptying of the stomach [93]. For the fourth investigation (ECO-Fed) horses were allowed access to free-choice alfalfa hay before and during the study period. Fresh water was available at all times. An IV catheter was placed on the morning of testing approximately 1 hour prior to the administration of the omeprazole to allow collection of the blood samples. When the investigation involved IV omeprazole catheter placement was repeated on the opposite jugular vein to facilitate administration of the drug. The catheter was removed immediately following administration of omeprazole.

A pre-administration blood sample was collected, the omeprazole administered and further blood samples collected from the catheter at T= 2 (IV only), 5 (IV only), 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135 min and 2.5, 3, 3.5, 4, 5, 6, 9 and 12 hours. Ten mL of blood was collected on each occasion with 4 mL transferred to a lithium heparin tube (Vacutainer®, BD, North Ryde, NSW, Australia). The samples were separated by centrifugation at 3,500 g for 10 min immediately following collection and the plasma frozen (-20 °C) within 30 min of collection.

2.4.3 Sample extraction

A protein precipitation extraction was performed; 50 µL of the sample was added to a 1.5 mL Eppendorf tube and 150 µL of a methanol: acetone mix at a ratio of 80:20 was added. The solution was then mixed by vortexing for 15 seconds, followed by centrifugation of the mixture at 15,000 g for 10 min. The supernatant was collected for UHPLC-MS analysis. Recovery of omeprazole for the extraction method was >95%.

2.4.4 Determination of plasma omeprazole concentration

A Nexera UHPLC coupled with LCMS-8030 triple quadruple mass spectrometer (Shimadzu Corporation, Tokyo, Japan) operating in positive electrospray ionization (ESI) mode was used for analysis with a reverse phase C18 column (Kinetex 1.7μ XB-c18 100A, size 50 x 2.1mm, Phenomenex, North Ryde, Australia) employed; the injection volume was 5 µL. The mobile phase consisted of Solvent A (10 mmol/L ammonium formate) and Solvent B (acetonitrile) in the following program: a gradient run of 40% B to 60% B for 1 min, a 0.1 min to 95% B and held for 1 min and then run at 5% B in an isocratic mode for 1
min. The flow rate was maintained at 0.4 mL min\(^{-1}\) at temperature of 40 °C. The drying gas was at 250 °C, the gas flow at 15 L/min, the nebulizing gas flow at 3 L/min, and the heating block at 400 °C. Nitrogen was used as the drying and nebulizing gas and the capillary voltage was 4.5 KV. Using direct flow injection analysis the omeprazole was optimized for solvent extraction in a positive mode with a MRM scan of precursor ion 346.2 and its product ion of 198.0. The dwell time was 50 ms for all. The chromatography method was validated by the manufacturer (Shimadzu Corporation, Sydney, NSW, Australia) using a stock standard of omeprazole diluted in acetonitrile with a lower limit of quantification of 0.001 μg/mL and a relative standard deviation of < 3% for AUC and drug concentration reported.

A calibration curve was prepared from a stock standard of omeprazole diluted in acetonitrile at 20 μg/mL with serial four fold dilutions performed using a methanol: acetone mix at a ratio of 80:20. The final calibration curve concentrations were 5, 1.25, 0.3125, 0.07812 and 0.019 μg/mL. Samples of each calibration curve concentration were frozen at -20 °C. To adjust for variability in conditions between runs, the same stock calibration curve solutions were used throughout. A 50 μL aliquot of each calibration curve concentration was allowed to warm to room temperature and then transferred to a 200 μL sampling tube for analysis. Calibration points were performed on three occasions (the beginning, middle and end) of each run. All data points were manually examined for validity as determined by the appearance of the chromatograph with valid data points, including the repeated samples for the calibration, then processed using the UHPLC post-run analysis software (Lab Solutions, Shimadzu Corporation, Tokyo, Japan) allowing correction for intra-run variability. Unknown samples were run in batches of 80-90 with a total run time of approximately 6 hours. The magnitude of intra-run variability was determined by calculating the coefficient of variation of the values for the replicates of the calibration curve samples determined at the beginning, middle and end of each run. Inter-run variability was determined by calculating the coefficient of variation between runs for each calibration point using average values within each run. Accuracy of the analysis was determined by calculating the coefficient of determination for the calibration curve using Microsoft Excel®'s (Microsoft, Redmont, WA, USA) graphing function.

2.4.5 Pharmacokinetic evaluation

The pharmacokinetic calculations were carried out by non-compartmental assessment of the data using an open source pharmacokinetic program (PK Solver, China
Maximum plasma concentration \((C_{\text{max}})\) and \(T_{\text{max}}\) were directly calculated from the data. The elimination rate constant \((\text{lambda}-z)\) was estimated by log-linear regression of concentrations observed during the linear phase of elimination, and the corresponding elimination \(t_{1/2}\) calculated as \(0.693/\lambda_{\text{-z}}\). The AUC vs. time curve \((\text{AUC}_{0-\infty})\) was calculated using the linear trapezoidal rule and oral \(F\%\) calculated from the ratio of the AUC, after oral and IV administration, indexed to their respective dose:

\[
F(\%) = \frac{(\text{AUC}_{\text{PO}} \times \text{Dose}_{\text{IV}})}{(\text{AUC}_{\text{IV}} \times \text{Dose}_{\text{PO}})} \times 100
\]

2.4.6 Data analysis

Data were analysed using GraphPad Prism® (GraphPad Software, La Jolla, CA, USA). The data were tested for normality using D’Agostino-Pearson omnibus K2 normality tests. The majority of data was non-Gaussian, so non-parametric statistical tests were used throughout. Oral \(F\%\), \(C_{\text{max}}\), \(T_{\text{max}}\) and \(\text{AUC}_{0-\infty}\) in ECO-fasted horses were compared to those in ECO-fed horses and PL-fasted horses using a Friedman test with Dunn’s multiple comparisons. Data from the oral formulations was pooled and the \(t_{1/2}\) for the oral formulations was compared to that for the IV-Fasted group using a Mann-Whitney \(U\) test. Data are presented as median (interquartile ranges (IQR)). Significance was set at \(P<0.05\).

2.5 Results

All horses successfully completed the study. A single adverse event was recorded with one dose of intravenous omeprazole accidently administered perivascularly (horse 5). No ill effects were observed as a result of the perivascular injection and the horse remained in the study. Due to the loss of the IV data set from horse 5, pharmacokinetic data for IV administration was available for only 11 horses. One horse (horse 2) demonstrated poor goodness of fit after subjective evaluation of the ECO-Fed data. Hence this horse was considered an outlier and was removed from comparisons between ECO-fed and ECO-fasted groups.

Intra-run variability, as determined by the relative standard deviation of replicate calibration curve samples within each run, was < 1% for all runs. Inter-run variability, as determined by the relative standard deviation of average intra-run values for each
calibration point, ranged from 5 - 14%. The coefficient of determination ($R^2$) for the calibration curve exceeded 0.99 for all runs.

The pharmacokinetics of omeprazole for the IV-Fasted group are summarized in table 2.1. Table 2.2 summarizes the pharmacokinetics for the ECO-Fasted, ECO-Fed and PL-Fasted groups. Median (IQR) plasma concentrations over time for the IV-Fasted, ECO-Fasted, ECO-Fed and PL-Fasted, groups are shown in figures 2.1 – 2.4, respectively.

The bioavailability of oral formulations is shown in table 2.3. Bioavailability was higher ($p=0.038$) in the ECO-Fasted group (21.5 [9.0-27.7] %) than the PL-Fasted group (10.1 [7.7-13.3] %). Similarly AUC$_{0-\infty}$ was higher in the ECO-Fasted group than the PL-Fasted group ($p=0.027$). $C_{\text{max}}$ did not differ between the ECO-Fasted and PL-fasted groups ($p=0.73$). No differences were present between the ECO-Fasted and ECO-Fed groups with regards to bioavailability, $C_{\text{max}}$, $T_{\text{max}}$ or AUC$_{0-\infty}$. Data from the oral formulations was pooled and the $t_{1/2}$ for the oral formulations (99.73 min [72.5-117.6]) was longer ($p<0.0001$) than the IV-Fasted group (35.5 min [33.4-39.1]).

### 2.6 Discussion

The findings following intravenous administration are consistent with a previous report wherein omeprazole has been described as having elimination half-life of approximately 30 min [74]. The longer half-life observed following oral administration is consistent with the flip-flop effect [97] and has not previously been described for omeprazole in the horse. The flip-flop effect primarily occurs in drugs that have a short half-life following intravenous administration. It occurs when the rate of absorption following extra-vascular administration becomes the predominant determinant of plasma concentrations, rather than the rate of elimination, as ongoing absorption of the drug interferes with the calculation of clearance and determination of half-life [97]. As such, the reported half-life following extra-vascular, in this case oral, should not be misinterpreted as a measure of drug clearance [97].

The bioavailability of the enteric-coated formulation examined in this study was higher than previously reported. In two previous studies evaluating bioavailability of an enteric-coated formulation serum concentrations of omeprazole were not detectable in 6/16 horses studied and in horses where the drug was detected an oral bioavailability of 6 – 14% was reported [4,78]. One likely reason for the discrepancy between the previous
and present studies is the method of analysis used. The older studies were performed over 20 years ago and the inability to detect omeprazole in serum despite adequate acid suppression in the horses [4,78] suggests that the limits of detection and/or the methodology used in the previous studies were much less sensitive than what is currently available. This is likely to significantly impact on the determination of AUC, which is primarily used to calculate bioavailability. Another factor that warrants consideration is the effect of formulation and the possibility that the formulation in the present study had superior bioavailability to that previously studied. The formulation previously studied was a preparation of enteric-coated granules encapsulated in gelatine formulated for human use [4,78], whereas the formulation in the current study consists of enteric-coated granules suspended into paste that is specifically formulated for use in horses. Direct comparison between formulations is needed before any conclusions regarding the effect of different formulations on bioavailability can be drawn.

The impact of feeding on the bioavailability of the enteric-coated formulation was minimal. This is in direct contrast to that previously reported for a buffered formulation of omeprazole where fasted horses had a 300% greater AUC [3]. Given that AUC is the primary determinant of the magnitude and duration of acid suppression achieved following omeprazole administration [66], the previous study reporting that feeding has a large impact the bioavailability of buffered formulations [3] suggests that there may be a significant impact of feeding on the efficacy of buffered formulations of omeprazole. This comment should be observed with caution as the previous study measured only a single time-point [3], but would still suggest that the effect of feeding on bioavailability may be partly dependent on formulation. As such, caution should be exercised in extrapolating the results of the present study to different formulations and further work is needed to define the interactions present between feeding, formulation and bioavailability.

A large degree of individual variation was observed with bioavailability in the ECO-Fasted group ranging from 7% to 56%. Three of the eleven horses for which bioavailability data was available appeared to absorb omeprazole poorly regardless of formulation or condition studied. In humans, variation in the efficacy of omeprazole has been reported and is primarily related to mutations in the CYP2C19 gene and changes in the rate of elimination [98]. Whether the poor bioavailability observed in these horses reflects increased clearance, as observed in humans, is unclear as the half-life following intravenous administration in the three horses with the lowest bioavailability represented a
wide range of values (26.4, 48.1 and 39.1 min for horses 2, 9 and 10, respectively) as shown in table 3. Alternatively factors that affect the rate of absorption may be important.

Regardless of the underlying reason, the finding that some animals in the current study repeatedly achieved only low plasma concentrations of omeprazole following oral administration is interesting. It potentially explains why healing of the squamous mucosa in only 70-85% of animals has consistently been observed in clinical trials [5–7,49,50] including one using the enteric coated formulation evaluated in the present study [8]. Healing of the squamous mucosa should readily occur if adequate acid suppression is achieved and it is tempting to speculate that horses that absorb omeprazole poorly may be less likely to respond clinically. Further work into the population pharmacokinetics of omeprazole and their impact on healing are justified.

The bioavailability of the plain omeprazole studied was approximately half that of the enteric-coated formulation. The median bioavailability of 10% was similar to that reported (10.5%) by the manufacturer of a generic plain omeprazole recently registered in the United Kingdom (Peptizole®) [99], although a greater range was observed in the present study (4-21% vs. 4-13% as reported by the manufacturer [99]). However, caution should be exercised in drawing direct comparisons between the formulation studied and the registered formulation as differences in the composition, purity, potency and stability are likely to exist.

The effect of feeding on the bioavailability of plain omeprazole was not investigated in the present study. At the time of study planning and execution buffered and enteric-coated formulations were the only forms commercially available. Accordingly, the primary objective of the study was to document the pharmacokinetics of enteric-coated omeprazole under different conditions as this was considered to be the most clinically relevant. The inclusion of plain omeprazole administered to fasted horses was intended to partially fill the void in the literature and to provide preliminary information for future studies. Further studies investigating the impact of feeding on the bioavailability of plain omeprazole would be beneficial in determining appropriate dosing strategies for that formulation.

In conclusion, the findings of the present study are similar to previous reports regarding the pharmacokinetics of intravenous omeprazole. In contrast, bioavailability of the enteric-coated formulation studied appears higher than previously reported and approximately twice that of plain omeprazole. Further work into comparative
bioavailability, the effects of feeding and the role of individual variability in therapeutic response are required.
Table 2.1 - Key pharmacokinetic parameters following administration of 0.5 mg/kg omeprazole intravenously.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Min</th>
<th>25% IQR</th>
<th>Median</th>
<th>75% IQR</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambda_2</td>
<td>l/min</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>t1/2</td>
<td>min</td>
<td>30.51</td>
<td>33.75</td>
<td>35.49</td>
<td>39.12</td>
<td>48.05</td>
</tr>
<tr>
<td>T_max</td>
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<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>C_max</td>
<td>µg/mL</td>
<td>1.02</td>
<td>1.53</td>
<td>1.82</td>
<td>2.06</td>
<td>2.51</td>
</tr>
<tr>
<td>C0</td>
<td>µg/mL</td>
<td>1.23</td>
<td>1.91</td>
<td>2.70</td>
<td>2.81</td>
<td>3.86</td>
</tr>
<tr>
<td>Clast_obs/C_max</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>AUC 0-t</td>
<td>µg/mL·min</td>
<td>23.14</td>
<td>32.90</td>
<td>38.33</td>
<td>40.34</td>
<td>49.06</td>
</tr>
<tr>
<td>AUC 0-∞</td>
<td>µg/mL·min</td>
<td>23.40</td>
<td>33.20</td>
<td>38.64</td>
<td>40.68</td>
<td>50.18</td>
</tr>
<tr>
<td>AUC 0-t/0-∞</td>
<td>%</td>
<td>97</td>
<td>98</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>AUMC 0-∞_obs</td>
<td>µg/mL·min⁻²</td>
<td>870.38</td>
<td>1212.26</td>
<td>1312.60</td>
<td>1691.34</td>
<td>2284.51</td>
</tr>
<tr>
<td>MRT 0-∞_obs</td>
<td>min</td>
<td>32.27</td>
<td>34.94</td>
<td>37.19</td>
<td>42.40</td>
<td>45.53</td>
</tr>
<tr>
<td>Vz_obs</td>
<td>(mg/kg)/(µg/mL)</td>
<td>4.56</td>
<td>4.79</td>
<td>5.12</td>
<td>6.98</td>
<td>9.14</td>
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<tr>
<td>Cl_obs</td>
<td>(mg/kg)/(µg/mL)/min</td>
<td>0.08</td>
<td>0.10</td>
<td>0.10</td>
<td>0.12</td>
<td>0.17</td>
</tr>
<tr>
<td>Vss_obs</td>
<td>(mg/kg)/(µg/mL)</td>
<td>3.17</td>
<td>3.59</td>
<td>3.63</td>
<td>4.40</td>
<td>6.36</td>
</tr>
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</table>
Table 2.2 - Key pharmacokinetic parameters following administration of 4 mg/kg of enteric-coated omeprazole to fasted horses (ECO-Fasted), enteric-coated omeprazole to fed horses (ECO-Fed) and plain omeprazole to fasted horses (PL-Fasted).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>ECO-Fasted</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>ECO-Fed</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>PL-Fasted</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>25% IQR</td>
<td>Median</td>
<td>75% IQR</td>
<td>Max</td>
<td>Min</td>
<td>25% IQR</td>
<td>Median</td>
<td>75% IQR</td>
<td>Max</td>
<td>Min</td>
<td>25% IQR</td>
<td>Median</td>
<td>75% IQR</td>
<td>Max</td>
</tr>
<tr>
<td>Lambda_z</td>
<td>1/min</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>t1/2</td>
<td>min</td>
<td>65.54</td>
<td>102.25</td>
<td>107.06</td>
<td>135.92</td>
<td>207.26</td>
<td>42.74</td>
<td>61.12</td>
<td>72.23</td>
<td>95.30</td>
<td>126.43</td>
<td>61.69</td>
<td>84.21</td>
<td>105.55</td>
<td>140.49</td>
<td>169.95</td>
</tr>
<tr>
<td>T_max</td>
<td>min</td>
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<td>55.00</td>
<td>60.00</td>
<td>100.00</td>
<td>110.00</td>
<td>20.00</td>
<td>50.00</td>
<td>80.00</td>
<td>100.00</td>
<td>180.00</td>
<td>20.00</td>
<td>30.00</td>
<td>30.00</td>
<td>30.00</td>
<td>110.00</td>
</tr>
<tr>
<td>C_max</td>
<td>µg/mL</td>
<td>0.14</td>
<td>0.24</td>
<td>0.40</td>
<td>0.67</td>
<td>1.90</td>
<td>0.08</td>
<td>0.29</td>
<td>0.40</td>
<td>0.71</td>
<td>1.16</td>
<td>0.08</td>
<td>0.22</td>
<td>0.25</td>
<td>0.29</td>
<td>0.47</td>
</tr>
<tr>
<td>Tlag</td>
<td>min</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Clst_l</td>
<td>µL/min</td>
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<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
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</tr>
<tr>
<td>AUC 0-t</td>
<td>µg/mL.min</td>
<td>23.71</td>
<td>32.79</td>
<td>51.45</td>
<td>70.76</td>
<td>163.10</td>
<td>8.82</td>
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<td>35.62</td>
<td>44.63</td>
</tr>
<tr>
<td>AUC 0-0-obs</td>
<td>µg/mL.min</td>
<td>24.97</td>
<td>33.28</td>
<td>52.90</td>
<td>71.06</td>
<td>161.28</td>
<td>8.97</td>
<td>34.00</td>
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<td>9.97</td>
<td>21.50</td>
<td>28.90</td>
<td>16.39</td>
<td>44.72</td>
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<tr>
<td>AUC 0-T</td>
<td>%</td>
<td>93</td>
<td>95</td>
<td>97</td>
<td>99</td>
<td>100</td>
<td>91</td>
<td>98</td>
<td>99</td>
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<td>100</td>
<td>92</td>
<td>96</td>
<td>98</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>AUMC 0-0-obs</td>
<td>µg/mL.min²</td>
<td>4225.51</td>
<td>7121.45</td>
<td>8183.45</td>
<td>8687.75</td>
<td>26829.31</td>
<td>1518.52</td>
<td>1697.71</td>
<td>5549.15</td>
<td>6362.13</td>
<td>15983.33</td>
<td>1518.52</td>
<td>1411.91</td>
<td>4236.14</td>
<td>5784.81</td>
<td>9444.50</td>
</tr>
<tr>
<td>MRT 0-0-obs</td>
<td>min</td>
<td>110.37</td>
<td>121.12</td>
<td>174.17</td>
<td>203.83</td>
<td>260.08</td>
<td>79.62</td>
<td>108.24</td>
<td>154.70</td>
<td>168.12</td>
<td>190.42</td>
<td>101.93</td>
<td>128.13</td>
<td>169.34</td>
<td>208.70</td>
<td>215.22</td>
</tr>
<tr>
<td>VS/F_obs</td>
<td>(mg/kg)/µL</td>
<td>2.32</td>
<td>9.07</td>
<td>11.65</td>
<td>19.12</td>
<td>41.80</td>
<td>4.11</td>
<td>4.81</td>
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<td>16.17</td>
<td>67.93</td>
<td>8.22</td>
<td>15.88</td>
<td>21.41</td>
<td>13.08</td>
<td>67.93</td>
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<tr>
<td>CL/F_obs</td>
<td>(mg/kg)/µL</td>
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<td>0.06</td>
<td>0.08</td>
<td>0.12</td>
<td>0.16</td>
<td>0.04</td>
<td>0.06</td>
<td>0.10</td>
<td>0.12</td>
<td>0.45</td>
<td>0.09</td>
<td>0.11</td>
<td>0.14</td>
<td>0.16</td>
<td>0.45</td>
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</table>
Table 2.3 - Bioavailability of enteric-coated omeprazole in fasted (ECO-Fasted) and fed (ECO-Fed) horses and plain omeprazole in fasted (PL-Fasted) horses.

<table>
<thead>
<tr>
<th></th>
<th>ECO-Fasted</th>
<th>ECO-Fed</th>
<th>PL-Fasted</th>
<th>IV t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse 1</td>
<td>22%</td>
<td>8%</td>
<td>20%</td>
<td>37.7</td>
</tr>
<tr>
<td>Horse 2</td>
<td>9%</td>
<td>n/a</td>
<td>4%</td>
<td>26.4</td>
</tr>
<tr>
<td>Horse 3</td>
<td>50%</td>
<td>4%</td>
<td>12%</td>
<td>33.8</td>
</tr>
<tr>
<td>Horse 4</td>
<td>8%</td>
<td>24%</td>
<td>10%</td>
<td>40.6</td>
</tr>
<tr>
<td>Horse 5</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Horse 6</td>
<td>22%</td>
<td>20%</td>
<td>13%</td>
<td>34.6</td>
</tr>
<tr>
<td>Horse 7</td>
<td>28%</td>
<td>33%</td>
<td>21%</td>
<td>35.5</td>
</tr>
<tr>
<td>Horse 8</td>
<td>56%</td>
<td>18%</td>
<td>5%</td>
<td>37.1</td>
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<td>Horse 9</td>
<td>9%</td>
<td>11%</td>
<td>8%</td>
<td>48.1</td>
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<td>Horse 10</td>
<td>7%</td>
<td>9%</td>
<td>8%</td>
<td>39.1</td>
</tr>
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<td>Horse 11</td>
<td>25%</td>
<td>14%</td>
<td>10%</td>
<td>33.4</td>
</tr>
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<td>Horse 12</td>
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<td>Min</td>
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<td>25% IQR</td>
<td>9%</td>
<td>9%</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>22%</td>
<td>16%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>75% IQR</td>
<td>26%</td>
<td>23%</td>
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<tr>
<td>Max</td>
<td>56%</td>
<td>33%</td>
<td>21%</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.1 - Plasma omeprazole concentrations following the administration of 0.5 mg/kg intravenously to 11 horses (IV-Fasted group). Data presented as median [IQR].
Figure 2.2 - Plasma omeprazole concentrations following the administration of 4 mg/kg of enteric-coated omeprazole to 12 fasted horses (ECO-Fasted group). Data presented as median [IQR].
Figure 2.3 - Plasma omeprazole concentrations following the administration of 4 mg/kg of enteric-coated omeprazole to 11 fed horses (ECO-Fed group). Data presented as median [IQR].
Figure 2.4 - Plasma omeprazole concentrations following the administration of 4 mg/kg of plain omeprazole to 12 fasted horses (PL-Fasted group). Data presented as median [IQR].
Chapter 3 - The effect of feeding on the pharmacokinetic variables of two commercially available formulations of omeprazole.

This chapter consists of a paper published as a short communication:


### 3.1 Prelude

An early study evaluating the pharmacokinetics of a buffered formulation of omeprazole reported that fasted horses had a 300% greater AUC compared to fed horses administered the same dose [3]. This is in contrast to the findings of the first study in the current thesis (reported in chapter 2) wherein feeding did not affect the bioavailability of an enteric coated formulations of omeprazole. The contrast between these findings suggests that the impact of feeding on bioavailability may be, at least in part, dependent on the method of protection used.

Considering this the objectives of this study were;

- To investigate the impact of formulation (enteric coated and buffered) and feeding on pharmacokinetic variables associated with the oral administration of omeprazole in the horse.

### 3.2 Abstract

The objectives of this study were to investigate the impact of formulation (enteric coated and buffered) and feeding on pharmacokinetic variables associated with the oral administration of omeprazole in the horse. Six Thoroughbred racehorses were studied in a cross-over design. Each received 2 g of an enteric coated or buffered formulation in both the fed and fasted state. Plasma omeprazole concentrations were determined by UHPLC-MS. The effects of feeding or formulation on AUC\(_{0-\text{inf}_{\text{obs}}}\), \(t_{1/2}\), \(T_{\text{max}}\) or \(C_{\text{max}}\) were not statistically significant. However, a wider than expected degree of variation was present and examination of the raw data suggests that an effect of feeding, wherein the bioavailability of omeprazole may be reduced in the fed animal, may be present. Further investigation in a larger population of animals to assess the factors that contribute to the wide degree of absorption observed is warranted.
3.3 Introduction

Omeprazole is widely used for the treatment of gastric ulceration in the horse. Despite its widespread use, little has been published on factors that affect the absorption of oral formulations of omeprazole to date, including the effect of feeding. An early study investigating the pharmacokinetics of a buffered formulation reported that, based on total AUC, feeding resulted in apparent decrease in absorption to one-third of the levels observed in fasted animals [3]. However, this study was complicated in that the horses in the fasted state had already received five doses of omeprazole, and were compared to horses in the fed state after their first and fourteenth doses [3]. In contrast, a recent study investigating the pharmacokinetics of a single dose of an enteric coated formulation of omeprazole reported that absorption in fed animals was not significantly reduced compared to fasted animals [100]. These findings suggested that the effect of feeding may be, at least in part, dependent on the formulation used. If present, such an effect would have significant implications for the dosing of different formulations of omeprazole under different clinical conditions. The objectives of this study were therefore to investigate the impact of formulation (enteric coated and buffered) and feeding on pharmacokinetic variables associated with the oral administration of omeprazole in the horse.

3.4 Materials and Methods

3.4.1 Animals & animal ethics

Six healthy, adult, privately owned Thoroughbred horses (aged 4 – 8 years, 455 - 510 kg BW) consisting of three male castrates and three females were used. During the studies horses were housed in a 12 m² stable bedded with wood shavings and fed as per the protocol for the allocated treatment (see below). The study was performed under an ethics permit from the New South Wales Department of Primary Industries (TRIM 14/836). Informed consent from the owner, or the trainer acting as an agent for the owner, was obtained at the time of enrolment to the study.

3.4.2 Group allocation and sequencing

Each horse received four treatments: Enteric coated – Fasted (ECO-Fasted), Enteric coated – Fed (ECO-Fed), Buffered – Fasted (BUFF – Fasted) and Buffered – Fed (BUFF – Fed). The formulations studied were chosen as they are the two leading selling
brands in Australia. Both formulations are registered for use in horses in Australia. The enteric coated formulation (Gastrozol®) studied was a 50 mg/mL paste formulation for oral administration. The buffered formulation (Omoguard®, CEVA, Glenorie, NSW, Australia) studied was a 370 mg/g paste formulation for oral administration. Horses were administered a total dose of 2 g (equivalent to 4 mg/kg for a 500 kg animal) in each treatment of the trial. A total dose was chosen over an individualized mg/kg dose as the presentation of one of the formulations precluded accurate fine tuning of the dose. Each horse received a different medication each week in a cross-over study. Based on a previously reported half-life following intravenous administration of approximately 30 minutes [74,100] a washout period of at least 1 week separated the treatments. Further, previous studies have documented that prior administration of the drug does not affect the pharmacokinetics of subsequent doses in the horse [3]. During the washout period horses were allowed access to pasture supplemented by good quality alfalfa hay.

Each horse was weighed on electronic scales (Accuweigh Equestrian Scales®) the day prior to each investigation and the amount of supplemental hay fed adjusted to ensure that the maximal deviation from their baseline bodyweight was ± 5 %. For two of the investigations (ECO-Fasted, and BUFF-Fasted) the horses were fasted for 16 hours to ensure adequate emptying of the stomach [93]. For the other two investigations (ECO-Fed and BUFF-Fed) horses were allowed access to free-choice alfalfa hay before and during the study period. Fresh water was available at all times. An IV catheter was placed on the morning of testing approximately 1 hour prior to the administration of the omeprazole to allow collection of the blood samples.

A pre-administration blood sample was collected, the omeprazole administered and further blood samples collected from the catheter at T = 15, 30, 45, 60, 75, 90, 105, 120 min and 2.5, 3, 3.5, 4, 5, 6 and 9 hours. Ten mL of blood was collected on each occasion with 4 mL transferred to a lithium heparin tube (Vacutainer®). The samples were separated by centrifugation at 3,500 g for 10 min immediately following collection and the plasma frozen (-20 °C) within 30 min of collection. Samples were processed within 6 weeks of collection and all analysis was completed within a 1 week period.

3.4.3 Sample extraction and determination of plasma omeprazole concentration

Sample extraction was performed using protein precipitation extraction and plasma omeprazole concentration was determined by UHPLC-MS analysis as recently described [100]. The magnitude of intra-run variability was determined by calculating the coefficient
of variation of the values for the replicates of the calibration curve samples determined at
the beginning, middle and end of each run. Inter-run variability was determined by
calculating the coefficient of variation between runs for each calibration point using
average values within each run. Accuracy of the analysis was determined by calculating
the coefficient of determination for the calibration curve using Microsoft Excel®'s graphing
function.

3.4.4 Pharmacokinetic evaluation

The pharmacokinetic calculations were carried out by non-compartmental
assessment of the data using an open source pharmacokinetic program (PK Solver) [96].
Maximum plasma concentration (C_{max}) and T_{max} were taken directly from the observed
data. The elimination rate constant (lambda-z) was estimated by log-linear regression of
concentrations observed during the linear phase of elimination, using a minimum of three
data points automatically selected by the program and confirmed by visual examination of
the plotted data. The corresponding elimination t_{1/2} was calculated as 0.693/lambda-z.
The AUC vs. time curve (AUC_{0–∞}) was calculated using the linear trapezoidal rule.

3.4.5 Data analysis

Data were analysed using GraphPad Prism® and R/R Commander® (McMaster
University, Hamilton, ON, Canada). The data were tested for normality using D’Agostino-
Pearson omnibus K2 normality tests. The data was non-Gaussian so it was log
transformed then analysed. A preliminary ANOVA was performed to assess whether order
of drug administration had an effect on any of the variables tested (T_{max}, C_{max} and AUC_{0–∞}).
A two-way repeated measures ANOVA with Sidak’s multiple comparisons test was then
performed with feeding (fed vs. fasted) and formulation (ECO vs. BUFF) as factors for
each variable. Significance was set at P<0.05. A power calculation was performed based
on the previously reported difference between fed and fasted states of 300% [3] and
suggested that 6 horses would provide a power of >80% to detect a difference between
the groups.

3.5 Results

All horses successfully completed the study. Median intra-run variability, was 3.2%
[IQR: 0.7 – 12.5%]. Median inter-run variability was 3.2% [IQR: 1.5 – 6.5%]. The median
coefficient of determination (R^2) for the calibration curves was > 0.99 [IQR: 0.96 – >0.99].

66
The key pharmacokinetic parameters for the four conditions studied are shown in table 3.1. Median (IQR) plasma concentrations over time for the ECO-Fasted, BUFF-Fasted, ECO-Fed and BUFF-Fed groups are shown in figures 3.1 – 3.4 respectively. When assessed visually both formulation and feeding appeared to influence the pharmacokinetic behaviour of the drug however, there was no statistically significant effect of feeding or formulation on AUC$_{0-\infty}$, $t_{1/2}$, $T_{\text{max}}$ or $C_{\text{max}}$. There was no effect of order of drug administration on any of the variables tested ($T_{\text{max}}$, $C_{\text{max}}$ and AUC$_{0-\infty}$).

3.6 Discussion

The results of the present study suggest that the effects of feeding on the absorption of an orally administered buffered formulation of omeprazole are less than previously described [3]. The magnitude of the effect observed for the enteric coated formulation is similar to a recent report [100].

No statistically significant differences were observed between the fed and fasted states for either formulation. However, examination of the raw data, in particular AUC$_{0-\infty}$, suggests that an effect may be present but that a wide degree of variation is present. The magnitude of variation was greater than anticipated and consequently the power of the study to detect a difference between the groups was reduced. Considering this; the authors propose that, although not statistically significant, the results of this preliminary study justify further investigation into the potential effects of feeding on the pharmacokinetics of different formulations of omeprazole in larger populations of animals. The wide degree of variation observed in bioavailability suggests that the investigation of population pharmacodynamic modelling is warranted.
Table 3.1 – Key pharmacokinetic parameters of omeprazole following the administration of 2 g of either an enteric coated (ECO) or buffered (BUFF) formulation of omeprazole orally in the fed and fasted state to six horses.

<table>
<thead>
<tr>
<th>Value</th>
<th>Unit</th>
<th>Fed</th>
<th>Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>25 IQR</td>
</tr>
<tr>
<td>Enteric-coated omeprazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambda_(\alpha)</td>
<td>l/min</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>t_{1/2}</td>
<td>min</td>
<td>24.46</td>
<td>46.16</td>
</tr>
<tr>
<td>T_{\text{max}}</td>
<td>min</td>
<td>30.00</td>
<td>30.00</td>
</tr>
<tr>
<td>C_{\text{max}}</td>
<td>µg/ml</td>
<td>0.09</td>
<td>0.20</td>
</tr>
<tr>
<td>AUC_{0-1}</td>
<td>µg/ml-min</td>
<td>10.58</td>
<td>12.17</td>
</tr>
<tr>
<td>AUC_{0-inf,obs}</td>
<td>µg/ml-min</td>
<td>10.67</td>
<td>12.93</td>
</tr>
<tr>
<td>AUC_{0-t/0-inf,obs}</td>
<td>%</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>MRT_{0-inf,obs}</td>
<td>min</td>
<td>73.54</td>
<td>79.30</td>
</tr>
<tr>
<td>Vz/F_{obs}</td>
<td>g/(µg/mL)</td>
<td>3.55</td>
<td>5.86</td>
</tr>
<tr>
<td>Cl/F_{obs}</td>
<td>g/(µg/mL)/min</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Buffered omeprazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambda_(\alpha)</td>
<td>l/min</td>
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<td>0.01</td>
</tr>
<tr>
<td>t_{1/2}</td>
<td>min</td>
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<td>57.20</td>
</tr>
<tr>
<td>T_{\text{max}}</td>
<td>min</td>
<td>30.00</td>
<td>30.00</td>
</tr>
<tr>
<td>C_{\text{max}}</td>
<td>µg/ml</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>AUC_{0-1}</td>
<td>µg/ml-min</td>
<td>4.04</td>
<td>5.68</td>
</tr>
<tr>
<td>AUC_{0-inf,obs}</td>
<td>µg/ml-min</td>
<td>4.24</td>
<td>6.32</td>
</tr>
<tr>
<td>AUC_{0-t/0-inf,obs}</td>
<td>%</td>
<td>89</td>
<td>94</td>
</tr>
<tr>
<td>MRT_{0-inf,obs}</td>
<td>min</td>
<td>57.04</td>
<td>78.17</td>
</tr>
<tr>
<td>Vz/F_{obs}</td>
<td>g/(µg/mL)</td>
<td>4.56</td>
<td>6.51</td>
</tr>
<tr>
<td>Cl/F_{obs}</td>
<td>g/(µg/mL)/min</td>
<td>0.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Figure 3.1 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of an enteric coated formulation of omeprazole to six fasted horses.
Figure 3.2 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of a buffered formulation of omeprazole to six fasted horses.
Figure 3.3 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of an enteric coated formulation of omeprazole to six fed horses.
Figure 3.4 - Median (IQR) serum concentrations over time for omeprazole following the administration of 2 g of a buffered formulation of omeprazole to six fed horses.
4.1 Prelude

To date little work has been done on the comparative pharmacokinetics of different formulations of omeprazole in the horse. The findings of first study of this thesis (as reported in chapter 2) supported the recommendation that omeprazole be protected in some form to prevent degradation by gastric acidity. However, little is known on the relative efficacy of different mechanism of protection, namely buffered pastes versus enteric coated formulations. A recent study no difference between the pharmacokinetics of a buffered formulation (GastroGard®) and an enteric coated formulation (Gastrozol®) although the doses used in this study were not identical. As such drawing firm conclusions is not possible as dose linear pharmacokinetics have not been demonstrated in the horse.

Considering this the objectives of the study were to;

- To investigate the relative pharmacokinetics of five commercially available formulations of omeprazole in the horse;

- To test for bioequivalence of four of the formulations against a reference formulation (GastroGard®).

4.2 Abstract

Omeprazole is widely used in the treatment of equine gastric ulcer syndrome. To date, little is known about the relative pharmacokinetics of the different formulations making comparisons between products difficult. The objectives of the study were to investigate the relative pharmacokinetics of five commercially available formulations of omeprazole in the horse, and to test for bioequivalence of four of the formulations using one of the formulations as a reference standard. Twelve mature Thoroughbred horses were fasted for 16 hours then administered 2 g of each formulation in a cross-over design. Serial blood samples were collected and plasma omeprazole concentration was
determined by UPLC-MS. No significant differences were present between three of the formulations and the reference formulation, while the fourth formulation had a lower $C_{\text{max}}$ and longer $T_{\text{max}}$ than the reference formulation. Bioequivalence against the reference formulation could not be demonstrated for any of the formulations tested. The findings of the study suggested that the method of protection utilised by different formulations of omeprazole (enteric coated granules vs. buffering) does not significantly alter the pharmacokinetics of the drug. Further work to establish bioequivalence is needed before direct comparisons can be drawn between different formulations.

4.3 Introduction

Omeprazole is widely used in the treatment of EGUS. It has been previously recommended that omeprazole requires some form of protection because exposure to acid in the stomach followed by alkalinisation in the small intestine renders the drug inactive before absorption can occur [11]. As such, commercial formulations of the drug typically incorporate some form of protection such as buffering with a highly alkaline medium, or the use of enteric coated granules suspended in a paste. A recent study reported the bioavailability of an enteric coated granule formulation (Gastrozol®) to be approximately twice that of a compounded, plain, unprotected omeprazole [100] supporting this recommendation.

The efficacies of the different methods of protection are, however, poorly investigated to date. A recent study compared the bioavailability of GastroGard®, which utilises a highly alkaline medium to buffer the omeprazole, to Gastrozol®, a formulation containing enteric coated granules suspended in a paste [69]. In that study, no difference in endoscopic healing of lesions was observed and the bioavailability of Gastrozol® was 1.26 times higher than GastroGard®, although the difference was not statistically significant as a wide 95% confidence interval (95% CI: 0.56 – 2.81) was present [69]. However, interpretation of the results was difficult as the dose of omeprazole used for GastroGard® of 4 mg/kg PO once daily differed from the dose of 1 mg/kg PO once daily used for Gastrozol®. Yet, to the authors’ knowledge, dose linear pharmacokinetics have not been demonstrated for omeprazole in the horse; it cannot be assumed that the pharmacokinetics of the two doses are equivalent, and the direct comparison of different doses is not appropriate.

Considering the current lack of information on the efficacy of methods of protection the primary objective of this study was to investigate the relative pharmacokinetics of five
commercially available formulations of omeprazole in the horse. A second objective of the study was testing for bioequivalence of four of the formulations against a reference formulation (GastroGard®).

4.4 Material and Methods

Five commercially available formulations of omeprazole were studied. GastroGard® was used as the reference formulation. Two buffered formulations, Omoguard® and Abgard® (Abler, Vanuatu), and two enteric coated granule formulations, Gastrozol® and BOVA Omeprazole Granules (BOVA Compounding, Caringbah, NSW, Australia) were used as comparison formulations. Details of the formulations including the method of protection utilised and concentration are shown in table 4.1.

GastroGard® was chosen as the reference formulation due to extensive evaluation with numerous studies documenting its efficacy in the treatment of squamous gastric ulceration at a dose of 4 mg/kg PO once daily [5–7,50,94,95]. The remaining formulations were chosen because of their availability commercially in either Australia (Gastrozol®, Omoguard®, BOVA Omeprazole Granules) or the Middle East (Abgard®). Where the formulations were not available in Australia (GastroGard® and Abgard®), they were imported on an APVMA special use permit.

4.4.1 Animals and animal ethics

Twelve healthy, adult, privately owned Thoroughbred horses (aged 3–13 years, 460–588 kg bodyweight (BW) consisting of six male castrates and six females were used. During the studies, horses were housed in a 12 m² stable bedded with wood shavings. Based on a previously reported t₁/₂ of approximately 30 min following IV administration [74,100], a washout period of at least 1 week separated the treatments during which time horses were allowed access to pasture supplemented by good quality alfalfa hay.

The study was performed under an ethics permit from the New South Wales Department of Primary Industries (TRIM 13/1125). Informed consent from the owner, or the trainer acting as an agent for the owner, was obtained at the time of enrolment to the study.
4.4.2 Group allocation and sample collection

Horses were blocked into two groups of six animals. The six horses within each block were randomised on week 1 with five of the horses receiving one of the medications and the sixth horse receiving no medication. The horses were subsequently managed in a cross-over design such that each treatment was administered to a single horse each week and one horse received no medication. At the completion of the six week studied period each horse had received one medication per week for five of the weeks and no medication in one of the weeks. The original six horses were then replaced by the subsequent block of six horses in an all-in/all-out manner.

The presentation of two of the formulations (Omoguard® and Abgard®) was not conducive to dose titration on an mg/kg basis. Instead each formulation was administered at a total dose of 2 g (equivalent to 4 mg/kg for a 500 kg horse) orally. To minimise week to week variation in BW, each horse was weighed on electronic scales (Accuweigh Equestrian Scales®) on a weekly basis and the amount of supplemental hay fed adjusted to ensure that the maximal deviation from their baseline BW was ± 5 %.

For all of the investigations the horses were fasted for 16 hours to ensure adequate emptying of the stomach [93]. Fresh water was available at all times. An IV catheter was placed on the morning of testing approximately 1 hour prior to the administration of the omeprazole to allow collection of the blood samples. A pre-administration blood sample was collected, the omeprazole administered and further blood samples collected from the catheter at T= 15, 30, 45, 60, 75, 90, 105 min and 2, 2.5, 3, 3.5, 4, 5, 6 and 8 hours. Ten mL of blood was collected on each occasion with 4 mL transferred to a lithium heparin tube (Vacutainer®). The samples were separated by centrifugation at 3,500 g for 10 min immediately following collection and the plasma frozen (-20 °C) within 30 min of collection.

4.4.3 Sample extraction and determination of plasma omeprazole concentration

Sample extraction was performed using protein precipitation extraction and plasma omeprazole concentration was determined by UHPLC-MS analysis as recently described [100]. The magnitude of intra-run variability was determined by calculating the coefficient of variation of the values for the replicates of the calibration curve samples determined at the beginning, middle and end of each run. Inter-run variability was determined by calculating the coefficient of variation between runs for each calibration point using
average values within each run. Accuracy of the analysis was determined by calculating the coefficient of determination for the calibration curve using Microsoft Excel®'s graphing function.

4.4.4 Pharmacokinetic evaluation

The pharmacokinetic calculations were carried out by non-compartmental assessment of the data using an open source pharmacokinetic program (PK Solver) [96]. Maximum plasma concentration (C<sub>max</sub>) and T<sub>max</sub> were directly calculated from the data by the software program. The elimination rate constant (lambda-z) was estimated by log-linear regression of concentrations observed during the linear phase of elimination, using at least three data points automatically selected by the program and confirmed by visual examination of the plotted data. The AUC vs. time curve (AUC<sub>0-∞</sub>) was calculated using the linear trapezoidal rule.

4.4.5 Data analysis

Data were analysed using a computer software program (R/R Commander®). Data was assessed for normality using the D'Agostino-Pearson omnibus normality test. The data was non-parametric and was log-transformed to allow parametric analyses. The effect of order of administration (before vs. after reference formulation) was evaluated using t-tests. A Bonferroni correction was applied to account for multiple comparisons. A repeated measures ANOVA with Dunnett’s post-test was performed to compare the AUC<sub>0-∞</sub>, C<sub>max</sub> and T<sub>max</sub> of the reference formulation (GastroGard®) versus the comparison formulations. Significance was set at P≤0.05. Summary statistics are expressed as mean (± SD) or geometric mean with corresponding 95% confidence intervals. Inter- and intra-run variabilities are reported as median [IQR].

Bioequivalence was calculated as per the committee for veterinary medicinal products guidelines for the conduct of bioequivalence studies for veterinary medicinal products [101]; for the comparison compound to be considered bioequivalent to the reference formulation (GastroGard®) the back-transformed 90% confidence interval for the ratio of the two treatment means must be contained within the limits 0.8-1.25. For each drug compared with the reference formulation, the hypothesis was that the ratio of the mean T<sub>max</sub>, C<sub>max</sub> and AUC<sub>0-∞</sub> of the reference and comparison drug lies between 0.8 (4/5) and 1.25 (5/4).
4.5 Results

All horses successfully completed the study and no adverse events were noted. Median intra-run variability for omeprazole concentration was 4.8% [IQR: 3.5 – 6.3%]. Median inter-run variability was 13.2% [IQR: 6.3 – 17.6%]. The median coefficient of determination ($R^2$) for the calibration curve was >0.99 [IQR: >0.99 - >0.99].

Figures 4.1 shows scatter plots for $T_{\text{max}}$, $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ for each of the formulations of omeprazole. Each of the comparison formulations $T_{\text{max}}$, $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ were compared with the reference formulation. There were no statistically significant differences in $T_{\text{max}}$, $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ between the reference formulation and comparison formulations for any variables, except between $T_{\text{max}}$ (P=0.029) and $C_{\text{max}}$ (P=0.036) of GastroGard® and Abgard®. The ratio and back-transformed 90% confidence interval between the comparison formulations and reference formulation for $T_{\text{max}}$, $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ are shown in Figure 4.2. Bioequivalence between the reference formulation and any of the comparison formulations could not be demonstrated. Horses that received Omoguard® after GastroGard® had a lower $T_{\text{max}}$ than those that received Omoguard® before GastroGard® (P=0.003).

4.6 Discussion

The results of the present study suggest that there may be modest pharmacokinetic differences present between some commercially available formulations of omeprazole in the horse. However, when compared with the reference standard formulation, the differences were not statistically significant for three of the formulations evaluated in the present study. The fourth formulation (Abgard®) was an exception with a lower $C_{\text{max}}$ and longer $T_{\text{max}}$ than the reference formulation (GastroGard®) observed. The clinical significance of this is unclear as AUC, which did not differ statistically between the two formulations, is considered to be the primary determinant of efficacy in humans [102]. Further Abgard® has previously been demonstrated to be efficacious in the treatment of squamous gastric ulceration in horses at a rate comparable to previous reports for GastroGard® [9].

The pharmacokinetic profiles of the enteric coated granule formulations (Gastrozol®; BOVA Omeprazole Granules) did not differ significantly from that of GastroGard®. This is consistent with a previous study wherein the bioavailability of Gastrozol® was 1.26 times higher than GastroGard® but the difference was not statistically significant as a wide 95%
confidence interval (95% CI: 0.56 – 2.81) was present [69]. Together, these results suggest that minimal, if any difference, exists between the pharmacokinetics of enteric coated granule formulations and buffered formulations. This is in contrast to plain, unprotected omeprazole which has been previously reported to have approximately half the bioavailability of an enteric coated granule formulation [100].

The inability to demonstrate a difference between enteric coated granule formulations and buffered formulations is interesting in light of recent clinical studies. Gastrozol® at a dose of 1 mg/kg PO once daily has recently been demonstrated to be non-inferior to the same formulation administered at the reference dose of 4 mg/kg PO once daily in the treatment of naturally occurring gastric ulceration in Thoroughbred racehorses [8]. The rates of endoscopic healing observed in that study were comparable to those previously reported for GastroGard® [5–7,50,94,95]. Similarly, no difference in healing of endoscopic lesions was observed in a trial comparing Gastrozol® at 1 mg/kg PO once daily against GastroGard® at 4 mg/kg PO once daily [69]. Given that a difference in pharmacokinetics between enteric coated granule formulations and buffered formulations could not be demonstrated in this study, these findings suggest that doses of buffered formulations of omeprazole lower than 4 mg/kg PO once daily may be efficacious. However, further studies are needed to document this as in a small study investigating the efficacy of a low dose of a buffered formulation of omeprazole (Abgard®) at 1.6 mg/kg PO once daily was inferior to the reference dose of 4 mg/kg PO once daily of the same formulation [10].

Despite the lack of a significant difference between the formulations being present bioequivalence could not be demonstrated. One potential explanation for this is the small sample size in the current study. Alternatively, true bioequivalence may not be present and as such care should be taken in directly extrapolating the results of studies, especially clinical studies, between formulations. Further studies comparing different formulations in larger samples of animals are warranted.

An effect of the order of administration on the T_{max} of Omoguard® was observed, wherein horses that received Omoguard® after GastroGard® had a lower T_{max} than those that received Omoguard® before GastroGard®. The potential for a type I error increases as the number of tests performed increases and the authors consider that it is likely that the finding is a statistical aberration as no biological reasons exists why such an effect should be present as it has previously been shown that prior administration of omeprazole
does not affect the pharmacokinetics of subsequent doses in the horse [3]. However, even if present, it is unlikely to be of clinical significance as AUC, not $T_{\text{max}}$, is considered the primary determinant of efficacy in humans [102].

The results of this study, in agreement with previous work, suggest that the pharmacokinetics of omeprazole as enteric coated granules in paste is similar to the pharmacokinetics of buffered formulations of omeprazole. However, bioequivalence could not be demonstrated for any of the formulations against the reference formulation and further studies with larger numbers of animals are needed to investigate whether the formulations studied are truly bioequivalent.
Table 4.1 - Trade names and formulation details of the five commercial omeprazole formulations studied.

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Method of protection</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GastroGard</td>
<td>Buffered</td>
<td>370 mg/g (407 mg/mL)</td>
</tr>
<tr>
<td>Omoguard</td>
<td>Buffered</td>
<td>370 mg/g (407 mg/mL)</td>
</tr>
<tr>
<td>Abgard</td>
<td>Buffered</td>
<td>370 mg/g (407 mg/mL)</td>
</tr>
<tr>
<td>Gastrozol</td>
<td>Enteric-Coated Granules in Paste</td>
<td>50 mg/ml.</td>
</tr>
<tr>
<td>BOVA Omeprazole Granules</td>
<td>Enteric-Coated Granules in Paste</td>
<td>100 mg/mL.</td>
</tr>
</tbody>
</table>
Figure 4.1 - Scatter plots of $T_{\text{max}}$, $C_{\text{max}}$ and Area-Under-the-Curve (AUC$_{0-\infty}$) for the five formulations tested (GastroGard (GG), Abgard, (AG), Omoguard (OG), BOVA Omeprazole Granules (BO), Gastrozol (GZ)). * denotes significantly ($P>0.05$) different from the reference formulation (GastroGard).
Figure 4.2 - The ratio and back-transformed 90% confidence interval between the comparison formulations (Abgard, (AG), Omoguard (OG), BOVA Omeprazole Granules (BO), Gastrozol (GZ)) and reference formulation (GastroGard (GG)) for $T_{\text{max}}$, $C_{\text{max}}$ and Area-Under-the-Curve (AUC$_{0-\infty}$). Dotted lines represent limits of bioequivalence.
Figure 4.3 - Mean (+ SD) plasma omeprazole concentration over time for five commercial formulations ((Abgard, (AG), Omoguard (OG), BOVA Omeprazole Granules (BO), Gastrozol (GZ)) and reference formulation (GastroGard (GG)).
Chapter 5 - Placement of an indwelling percutaneous gastrotomy (PEG) tube for the measurement of intra-gastric pH in two horses.

This chapter consists of a paper published as:


5.1 Prelude

As discussed earlier; the methodology used to investigate the pharmacodynamics of omeprazole in the earlier studies evaluating the use of omeprazole in the horse may not be truly reflective clinical conditions. Specifically the need to fast horses for the collection of gastric fluid may change the subsequent pH measurements obtained. More recently the use of pH probes fitted in a retrograde manner into indwelling gastric cannulas has been described [11]. This approach offers several advantages, most notably the ability to investigate the pharmacodynamics of omeprazole, and other acid suppressive agents, under normal dietary management. A downside of the later technique is the need for general anaesthesia and significant surgical skill to place the indwelling catheters. Most recently the endoscopic assisted placement of indwelling percutaneous gastrotomy (PEG) tubes has been described in the horse [103]. This combination of these two techniques is appealing as an alternative means of measuring intra-gastric pH in the horse.

The objective of the present study was;

- To describe a method of intra-gastric pH measurement using a pH probe that is fitted in a retrograde manner into an endoscopically placed, indwelling PEG in the horse.

5.2 Abstract

Intra-gastric pH monitoring is an important tool in the validation of acid suppressive drugs. To date, several methods of monitoring intra-gastric pH in the horse have been described. However significant limitations are present in the existing models. This case report describes the placement of a PEG tube secured with a button gastropexy in the standing horse through which the pH of the ventral stomach can readily be measured under both fed and fasted conditions.
5.3 Introduction

Suppression of gastric acid production is a cornerstone of the treatment of EGUS with omeprazole the drug most commonly used for this purpose. Early studies into the efficacy of omeprazole suggested that, using enteric coated omeprazole pellets in gelatine capsules, doses as low as 1.4 mg/kg BW given via nasogastric tube result in effective acid suppression for a minimum of 27 hours [4]. More recently it has been reported that the duration of acid suppression achieved following the oral administration of 4 mg/kg BW of a buffered formulation of omeprazole may result in a duration of acid suppression as short as 11 hours [11]. Potential reasons for these discrepancies include variation in the bioavailability of the different formulations and the reliability of the models used to study intra-gastric pH.

Recent studies have suggested that minimal, if any, differences are present between the bioavailability of enteric coated granule formulations and buffered formulations of omeprazole in horses. In a clinical study evaluating the efficacy of two formulations of omeprazole in horses, the bioavailability of an enteric coated granule formulation was 1.26 times higher than the comparison buffered formulation, although the difference was not statistically significant as wide 95% confidence intervals (95% CI: 0.56 – 2.81) were present [69]. In a study by the authors’ research group the bioavailability of two enteric coated granule formulations was tested against a reference buffered formulation (GastroGard®). No significant difference was present between $C_{\text{max}}$, $T_{\text{max}}$ and AUC for either formulation [104]. Together, these findings suggest that minimal, if any, differences are present between the pharmacokinetics of enteric coated granule and buffered formulations of omeprazole in horses. Direct comparison between the studies of Jenkins et al (1992) and Merritt et al (2003) is not possible; however the lack of differences in pharmacokinetic between currently available formulations suggests that alternative reasons, other than formulation, for the discrepancy in efficacy discussed above warrants consideration.

A limiting factor in any study of gastric acid suppression is a suitable model to accurately and reliably measure intra-gastric pH over time. Early studies used fasted horses to allow collection of gastric fluid [4,78], but this may affect the bioavailability of buffered omeprazole. One study showed fasted horses had a three-fold increase in bioavailability compared to fed horses [3]. In contrast, Merritt et al (2003) reported a model in which pH probes were fitted in a retrograde manner through surgically implanted
gastric cannulas. This method allowed intra-gastric pH to be monitored in horses under fed or fasted conditions and without interruption to their normal feeding behaviour and, therefore, is more clinically relevant. However, the need for general anaesthesia and advanced surgical skills for the placement of the gastric cannula could be considered as disadvantages of this model.

The purpose of this report is to describe a method of intra-gastric pH measurement using a pH probe that is fitted in a retrograde manner into an endoscopically placed, indwelling PEG in the horse.

5.4 Animals and animal ethics

Two healthy, male adult (505 kg and 540 kg; aged 8 and 10 years) Thoroughbred horses were used. Both animals had been in the investigators’ possession for a minimum of 12 months prior to use in the study, during which time they had received routine health care and remained healthy. Clinical examination was performed on both horses prior to use in the study and was unremarkable. During the studies the horses were housed individually in 16 m² stables bedded with wood shavings and fed as per the protocol for the allocated treatment (see below). The study was performed under an ethics permit from the New South Wales Department of Primary Industries (TRIM 14/981).

5.5 Materials and Methods

Horses were fasted for 20 hours and withheld from water for 2 hours prior to gastroscopy. Procaine penicillin (Propercillin®, Troy Laboratories, Glendenning, NSW, Australia) (25,000 IU/kg BW intramuscularly) was administered 2 hours prior to gastroscopy. Flunixin meglumine (Flunixon Injection®, Norbrook Laboratories, Tullamarine, VIC, Australia) (1.1 mg/kg BW IV) and gentamicin (Gentam 100®, Troy Laboratories, Glendenning, Australia) (8.8 mg/kg BW IV) were administered at the time of sedation. The horses were sedated with detomidine (Dozadine®, Virbac, Milperra, NSW, Australia) (10-20 µg/kg IV) and gastroscopy was performed using a 3.3. meter gastroscope (Portascope®, Portascope.com, Bradenton, FL, USA) as previously described [105]. Following introduction of the gastroscope the stomach was insufflated with air until fully distended.

A “button gastropexy” similar to the abomasal toggle technique described in cattle [106] was then performed using the following method; the stomach was identified on the
left-hand side of the horse at the 11th inter-costal space by percutaneous ultrasound and the surrounding area of skin aseptically prepared. A 2.1 x 133 mm (14 G x 5.25") catheter (Angiocath®, BD, North Ryde, NSW, Australia) was inserted through the skin and into the stomach until it could be observed gastroscopically. Once the location of the catheter was visually confirmed within the stomach a 3 m length of high tensile (40 lb.) fishing line was passed through the catheter from the outside and into the stomach (figure 5.1). Biopsy forceps were used to grasp the fishing line and draw it into the gastroscope. The gastroscope was then withdrawn, drawing the fishing line out of the nostril. A generic 15 mm wide button was then attached to the fishing line and carefully secured.

The catheter was withdrawn from the body wall and gentle traction was placed on the fishing line drawing the button back through the nostril and down the oesophagus and into the stomach. Once firm pressure was felt, suggesting that the button was in place against the body wall, the gastroscope was reinserted into the stomach to confirm the positioning of the button against the stomach wall (figure 5.2). A second button was placed approximately 3 – 5 cm away from the first button using the same technique. The ends of the two lines were then tied together under gentle traction against the body wall with a small plastic plate placed between the suture and the skin to offset the tension (figure 5.3).

Once the gastropexy was placed, a 20 Fr PEG tube (Mila International, Erlanger, KY, USA) was then placed using a similar technique. A similar PEG technique has previously been described; without first creating a gastropexy [103]. However, in pilot investigations using this technique the authors of this study twice experienced displacement of the PEG tube since it was not adequately secured within the stomach. In a further modification to the previous described technique, and to further reduce the risk of PEG tube displacement, a generic 15 mm nylon washer was placed over the PEG tube prior to placement such that it sat between the end of the PEG tube and body wall as shown in figure 5.4. Figure 5.3 shows the gastropexy and PEG tube in place externally. A video demonstrating placement of the button gastropexy and PEG tube is available at https://dl.dropboxusercontent.com/u/13295715/Movie%20-%20Tube%20Placement.mp4.

Following the procedure, horses were returned to their stalls and allowed to recover from sedation before being fed as per their normal routine. Procaine penicillin (25,000 IU/kg BW IM twice daily), gentamicin (8.8 mg/kg BW IV once daily) and flunixin meglumine (1.1 mg/kg BW IV twice daily) were continued for three days at which point in time the
horses were changed to doxycycline (Bova Compounding, Caringbah, NSW, Australia) (10 mg/kg PO twice daily) and phenylbutazone (P-Butazone®, Virbac, Milperra, NSW, Australia) (2.2 mg/kg PO once daily) for a further 7 days. Horses were monitored by full clinical examination, including examination of the gastropexy/PEG tube site, three times per day for the first two weeks. Further, examinations, such as ultrasound and/or abdominocentesis, were not performed as they were not deemed indicated based on the clinical condition of the horses. On-going monitoring consisted of twice daily monitoring of general demeanour, appetite and temperature as well as examination of the gastropexy/PEG tube site.

Three weeks following placement of the PEG tube the position of the tubes was confirmed gastroscopically. In both horses the tube was located within the ventral glandular fundus approximately 10 - 15cm below the margo plicatus of the greater curvature as shown in figure 3. At this point a pH probe (Comfortec PLUS®, Sandhill Scientific, Highlands Ranch, CO, USA) was fitted retrograde through the PEG tube with its location confirmed endoscopically as shown in figure 5.5. The probes contained two measurement points, 5 cm apart. The location of measurement point 1 was approximately 1 - 2 cm from the glandular mucosa while the second measurement point (measurement point 2) sat 5 cm deeper within the glandular portion of the stomach. The probe’s insertion distance into the PEG tube was noted for future placement which was performed blindly using the known insertion distance.

The ability of the model to monitor changes in gastric pH in horses on different feed types was then investigated using a cross over study design. The horses were adapted to one of two diets over a period of one week; a hay only diet (HAY) or a high grain/low fibre (HG/LF) diet. The HG/LF diet consisted of 1% BW each of grain and hay per day, divided equally into two meals fed at 10 am and 6 pm to mimic the management of a racehorse in training. The HAY diet consisted of ad libitum access to oaten/rye grass hay.

Once adapted to their respective diet the intra-gastric pH of each horse was then monitored for a period of 5 consecutive days. To achieve this; the pH probe was attached to a continuous data logger (ZepHr®, Sandhill Scientific, Highlands Ranch, CO, USA). Data recording commenced at 8 am and continued until 7 am the following morning (23 hours). Mean, median, minimum and maximum pH and the % time below a pH of 4 was reported directly from the software program. New probes were used for each study period and each probe was calibrated each day as per the manufacturer’s instructions. The
probe was then inserted the previously determined distance and secured. Following the 5
day data collection period each horse was then transitioned to the other diet over a period
of one week and the 5 day recording period was repeated on the other diet. Due to the
small number of animals and data points collected statistical analysis was not attempted.
Instead the results for each variable are presented descriptively in table 1 as median
(IQR).

5.6 Results

The procedure was well tolerated by both horses. Both horses had a mild fever
(<39.0 °C) within the first 48 hours following the procedure but retained a normal appetite,
and had otherwise normal clinical parameters. Forty-eight hours following the
discontinuation of the antimicrobials on day 10 one horse developed mild, generalised
swelling of the surgical site and a fever (up to 39.0°C). Antimicrobial therapy with
doxycycline (10 mg/kg PO twice daily) was reinitiated for a further 7 days. The horse
responded within 24 hours and no further episodes of fever were observed. At the time of
writing the tubes have been maintained in place for 6 months without further complication,
during which time the horses have been used repeatedly for intra-gastric pH measurement
as part of on-going studies.

Typical plots of pH over time for the HG/LF and HAY diets are shown in figures 5.6
and 5.7. Spikes in the recorded pH were typically seen following the morning feeding at
10 am in the HG/LF diet with a less consistent effect observed following the 6 pm feeding.
In the HAY diet the pH typically remained low throughout the measurement period with
less variation observed.

Table 5.1 summarises key pH values and the percentage of time below a pH of 4
for five consecutive days of pH measurement (measured from 8 am to 7 am) for two
horses under the two different dietary conditions.

5.7 Discussion

This report describes the successful placement of a permanent indwelling PEG tube
in the standing horse through which intra-gastric pH measurement can readily be
performed. Beyond the requirement for a gastroscope, no specialised equipment is
required and the technique is technically relatively simple with no specialised surgical skill
required. The procedure was well tolerated by the two horses, both in the short and long
term. Minor complications were observed and further evaluation in a larger number of horses is warranted to further document the safety of the procedure.

The ability to continuously record intra-gastric pH without altering the horse’s normal eating behaviour provides a useful model for investigating the efficacy of omeprazole and other acid suppressive drugs, which are the cornerstone of the management of EGUS, under clinically relevant conditions. Further, the location of the probe within the ventral region of the stomach may provide useful information in investigating the poor response of lesions within the glandular mucosa to omeprazole monotherapy as recently reported [8–10].

The model is similar to a gastric cannulation model previously used in a number of studies including those investigating the effect of pasture turnout vs. stall housing on gastric pH [40] and the efficacy of omeprazole in suppressing gastric acidity [11]. The primary advantage of the current model is the ability to place the PEG tube in the standing horse, negating the need for general anaesthesia. The authors consider that the greatest strength of both the previously reported gastric cannulation model and the current PEG tube technique is that the location of the probes within the stomach is known. This is in contrast to another model that has been described to measure ventral gastric pH that utilises a mercury weighted balloon [71]. This method can be performed in the standing horse but the final location of the pH probe is unknown. Further, the use of mercury presents significant health risks to the operator making this model undesirable.

The different pH profiles observed between the two diets is interesting and warrants discussion. As shown in figure 6 a spike in pH of approximately 1 – 3 hours duration was typically observed following morning, and to a lesser degree evening, feeding in the HG/LF group whereas the intra-gastric pH in the HAY group (figure 7) remained relatively stable with a pH of < 2. Reflective of this, the median percent time that the pH was below 4 appeared lower with the HG/LF diet than the HAY diet. The number of animals in this study is too small to draw conclusions, but further investigation into the effect of diet on ventral gastric pH in a larger number of animals appears warranted.
Table 5.1 – Summarised values for five 23 hour periods (8 am – 7 am the following day) for pH and the percentage of time below a pH of 4 recorded over five consecutive days in two horses under two different dietary conditions; ad libitum hay (HAY) and a high grain/low-fibre diet (HG/LF) consisting of 1% each of grain and hay divided into two meals. Measurement was performed at two points. Measurement point 1 was within 1–2 cm of ventral glandular mucosa. Measurement point 2 was 5 cm distal to measurement point 1. Data shown as median (IQR).

<table>
<thead>
<tr>
<th>Horse</th>
<th>Diet</th>
<th>Min pH</th>
<th>Max pH</th>
<th>Mean pH</th>
<th>Median pH</th>
<th>% time &lt;4</th>
<th>Min pH</th>
<th>Max pH</th>
<th>Mean pH</th>
<th>Median pH</th>
<th>% time &lt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HAY</td>
<td>0.99 [0.78;1.05]</td>
<td>6.20 [5.67;6.42]</td>
<td>1.78 [1.61;1.92]</td>
<td>1.63 [1.60;1.68]</td>
<td>99.8 [99.2;100]</td>
<td>0.97 [0.89;0.98]</td>
<td>6.04 [5.96;6.45]</td>
<td>1.88 [1.77;1.91]</td>
<td>1.87 [1.78;1.90]</td>
<td>99.8 [99.8;99.9]</td>
</tr>
<tr>
<td>1</td>
<td>HG/LF</td>
<td>0.85 [0.00;0.86]</td>
<td>6.02 [5.81;6.45]</td>
<td>2.32 [2.20;2.49]</td>
<td>2.05 [1.85;2.08]</td>
<td>91.3 [84.1;93.9]</td>
<td>0.18 [0.09;0.33]</td>
<td>6.40 [6.25;6.84]</td>
<td>2.12 [1.95;2.30]</td>
<td>1.64 [1.60;1.92]</td>
<td>87.0 [81.4;90.1]</td>
</tr>
<tr>
<td>2</td>
<td>HAY</td>
<td>0.94 [0.72;1.06]</td>
<td>4.73 [4.30;5.17]</td>
<td>1.67 [1.45;1.72]</td>
<td>1.61 [1.44;1.72]</td>
<td>100 [100;100]</td>
<td>0.81 [0.72;0.95]</td>
<td>6.15 [5.65;6.73]</td>
<td>1.88 [1.84;1.98]</td>
<td>1.89 [1.84;1.94]</td>
<td>99.9 [99.7;99.9]</td>
</tr>
<tr>
<td>2</td>
<td>HG/LF</td>
<td>0.52 [0.20;0.75]</td>
<td>5.56 [5.33;5.71]</td>
<td>1.93 [1.89;1.93]</td>
<td>1.61 [1.55;1.63]</td>
<td>92.2 [89.1;93.0]</td>
<td>0.54 [0.21;0.55]</td>
<td>6.20 [5.88;7.15]</td>
<td>1.99 [1.57;2.00]</td>
<td>1.66 [1.43;1.66]</td>
<td>91.0 [90.2;97.1]</td>
</tr>
</tbody>
</table>
Figure 5.1 - A gastroscopic image taken following transcutaneous insertion of a 14 G x 5.25” catheter into the stomach. A length of high tensile (40 lb.) fishing line is then passed through the lumen of the catheter and into the stomach.
Figure 5.2 - A gastroscopic image showing the positioning of the button within the ventral glandular mucosa below the greater curvature (left of image) and looking towards the lesser curvature and pyloric antrum.
Figure 5.3 - An image showing placement of the percutaneous gastrotomy (PEG) tube (top) and button gastropexy (bottom) at between 11th and 12th ribs.
Figure 5.4 - A gastroscopic image showing final placement of the button gastropexy and percutaneous gastrostomy (PEG) tube. Prior to PEG tube insertion a 25 mm nylon washer is placed over the shaft of the PEG tube to provide additional security against the stomach wall.
Figure 5.5 - A gastroscopic image showing final placement of the pH probe with two measurement points 5 cm apart. The first measurement point is located approximately 1–2 cm from the surface of the mucosa. The second measurement point sits freely within the ingesta/fluid contents of the ventral stomach.
Figure 5.6 - An example of a trace of continuous pH measurement of the ventral stomach over a 23 hour period in a horse (horse 1 – day 1) 6 receiving a high grain/low fibre (HG/LF) diet consisting of 1% each of grain and hay divided equally into two meals fed at 10:00 am and 6:00 pm. The top trace is from measurement point 1 and the lower trace is from measurement point 2.
Figure 5.7 - An example of a trace of continuous pH measurement of the ventral stomach over a 23 hour period in a horse (horse 1 – day 3) receiving a diet consisting of ad libitum hay. The top trace is from measurement point 1 and the lower trace is from measurement point 2.
Chapter 6 – The effect of dose and diet on the pharmacodynamics of omeprazole in the horse.

This chapter consists of a paper submitted to the Equine Veterinary Journal as:


6.1 Prelude

The findings of the earlier studies in this thesis were primarily focused on the influence of diet and formulation on the pharmacokinetics of omeprazole in the horse. The findings of chapter 4 suggested that the impact of formulation was minimal. However, bioequivalence between commercially available formulations could not be determined. As such the authors considered it appropriate to select GastroGard® as the formulation for evaluation in the final study since it has historically been the most widely used formulation globally.

Although diet could not be shown to be significantly influenced in the studies reported in chapter 2 or 3, the authors felt that the raw data and previously published literature suggested that an effect may be present. As such further investigation was warranted and two diets, representing difference ends of the clinical spectrum, were included for evaluation. Lastly, the effect of dose has been poorly described with no reports of direct comparisons between doses published.

Considering this the objective of the study was to use the model for intra-gastric pH measurement reported in chapter 5 to:

- Investigate the effect of two diets, representing different ends of the clinical spectrum on the pharmacodynamics of GastroGard® in the horse; and
- Investigate the effect of two doses on the pharmacodynamics of GastroGard® in the horse.

6.2 Abstract

Conflicting data is presented in the current literature regarding the efficacy of omeprazole for supressing gastric acidity in the horse. The objective of this study was to investigate the duration of intra-day acid suppression achieved with two doses of
omeprazole under two different dietary conditions using a 4-way cross-over design. Six adult, Thoroughbred horses instrumented with percutaneous gastrotomy tubes were used. Intra-gastric pH was measured for continuous 23 hour periods (8am – 7am) for 6 consecutive days (days 0-5). Baseline data was recorded on day 0 and omeprazole was administered on days 1-5. Two doses (1 mg/kg and 4 mg/kg PO once daily) and two diets (a high grain/low fibre (HG/LF) and ad libitum hay (HAY) diet) were studied. Data for the percent time pH was above 4 (%tpH>4) and median intra-day pH was reported for two measurement points and analysed using generalized estimating equations. An effect of both diet and dose was evident with mean %tpH>4 and mean median intra-day pH typically higher at the higher (4 mg/kg) dose and in HG/LF diet. The overall efficacy of omeprazole in raising intra-gastric pH was good under the HG/LF conditions but relatively poor in the HAY diet. A cumulative effect of dosing, not previously reported in the horse, was observed. The overall efficacy of omeprazole in raising ventral gastric pH was less than previously reported. Further, the present study suggests that both dose and diet may play a role in the efficacy of omeprazole in the horse. As such the use of singular dosing recommendations that encompass all horse types and management conditions may not be appropriate. Instead, dosing recommendations that take into account the diet of the horse may be advantageous.

6.3 Introduction

Equine Gastric Ulcer Syndrome (EGUS) is a common disease of horses worldwide. Recently the terminology has been refined to recognise Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) as separate entities [1]. Regardless of the lesion type omeprazole is recommended as the treatment of choice [1] and it is widely used for this purpose. Early studies investigating the pharmacodynamics of omeprazole in the horse suggested that it has a 24 hour duration of activity following enteral administration [3,4]. Its efficacy in the treatment of ESGD in a number of clinical studies [5–7] appeared to support this conclusion.

The results of these early studies were recently questioned by a study that measured intra-gastric pH of horses on an ad libitum hay diet using pH probes inserted retrograde into an indwelling gastric cannula [11]. In this study [7], the commercially-available formulation GastroGard® was compared to three compounded formulations of omeprazole with intra-gastric pH of the ventral stomach continuously recorded for 24 hour periods in horses fed ad libitum hay supplemented with a small grain meal twice daily.
Although GastroGard® was the most efficacious of the treatments studied, the time mean pH was above 4 was only 14 and 11 hours on days 2 and 7 of treatment, respectively. This is in contrast to the findings of earlier studies [2,3], but whether this was an effect of feeding or the formulation studied was not established.

The possibility that the duration of acid suppression achieved under clinical conditions may be less than originally reported in experiment studies is further supported by recent studies where the EGGD treatment response was only 25% with 28-35 days of omeprazole therapy at 4.0 mg/kg PO once daily in direct contrast to ESGD lesion healing rate of 78% in the same studies [8–10]. One of the possible explanations for this lower response rate is that the duration of intra-day acid suppression required for healing of ESGD may less than that required for healing of EGGD. As such, ≤ 14 hours of acid suppression may be adequate for ESGD healing, while EGGD healing may require acid suppression of a longer duration.

The objective of this study was to further investigate the duration of intra-day acid suppression achieved with two doses of omeprazole under two different dietary conditions using a recently described gastric cannulation model [107] that allows continuous monitoring of gastric pH under normal feeding conditions.

6.4 Materials and Methods

6.4.1 Horses

Six (3 male, 3 female) healthy Thoroughbred horses (aged 7 to 14 years, 480 – 565 kg BW) were used. Horses were housed in 16 m² stables and bedded on wood shavings throughout the study. Horses were feed a rye grass/lucerne hay mix ad libitum that was supplemented with a commercial feed pellet as required to maintain body condition throughout the study period unless otherwise dictated by the diet under investigation.

Each horse was instrumented with a percutaneous gastrotomy tube (PEG) as previously described [107]. The location of the PEG tube was confirmed by gastroscopy to be within the ventral glandular fundus approximately 10 – 15 cm below the margo plicatus adjacent to the lesser curvature in each horse.

6.4.2 Group allocation

Horses were assigned to treatment pairs with one pair studied each week in a 4-way cross over design. Two diets were evaluated; a hay only diet (HAY) or a high
grain/low fibre (HG/LF) diet. The HG/LF diet consisted of 5 kg grain (40% protein pellets (Equine Mare and Foal™, Vella Stock Feeds, Plumpton, NSW, Australia) and 60% sweet feed (Robank Non-Oat Custom Mix™, Robank, Ebenezer, NSW, Australia)) and 5 kg hay per day, divided equally into two meals fed at 10 am and 6 pm to represent typical dietary management of a racehorse in training [80]. The HAY diet consisted of \textit{ad libitum} access to a rye grass/lucerne hay mix. Horses were adapted to the diets over a period of at least 1 week prior to the commencement of data collection. Water was available \textit{ad libitum}. The remaining 4 horses rested and, where appropriate, transitioned between test diets.

6.4.3 \textbf{Administration of medication}

Two doses of omeprazole (GastroGard®) were evaluated; 1 mg/kg PO once daily and 4 mg/kg PO once daily. To ensure accurate dosing each horse was weighed on electronic scales (Accuweigh Equestrian Scales®) on day -1. The omeprazole was administered using the graduated dose markings on the syringe, rounding up to the nearest 50 pound (22.7 kg) dose. Medications were administered at 8 am (2 hours prior to feeding in the HG/LF diet).

6.4.4 \textbf{Gastric pH measurement}

Once adapted to their respective diet the intra-gastric pH of each horse was then monitored for a period of 6 consecutive days. To achieve this; the pH probe was attached to a continuous data logger (ZepHr pH®) and calibrated prior to placement each day as per the manufacturer’s instructions. The probe was then inserted a previously determined distance and secured as previously described [107]. Data was recorded at 2 points; measurement point 1 was located approximately 10 – 20 mm from the glandular mucosa and measurement point 2 was 5 cm further within the stomach as shown in figure 6.1 [107]. Data recording commenced at 8 am and continued until 7 am (23 hours). Mean intra-day, median intra-day, minimum and maximum pH and the \% time below a pH of 4 was reported directly from the data logger’s software program. The \% time above a pH of 4 (\%t>pH4) was calculated using the following equation: \% time above a pH = 100\% - \% time below a pH of 4. Baseline data was recorded on day 0 with the medication administered on days 1 – 5.
6.4.5 Data analysis

Data were assessed for normality. Distributions and correlations were screened graphically and using Pearson's or Spearman's correlation coefficient, with coefficients <0.4 regarded as poor, 0.4–0.7 moderate and >0.7 good [108]. Summary statistics are reported as estimated marginal means. Differences between groups over time were assessed using generalized estimating equations (GEE) utilizing the 'genlin' command in SPSS (www.ibm.com/software/au/analytics/spss) with ARIMA (Autoregressive Integrated Moving Average) selected as the optimal covariance structure. A GEE is conceptually similar to repeated measures ANOVA, in that it accounts for repeated measures on individual horses. GEE can be used when there are missing data points, and provides a more realistic covariance structure than ANOVA which assumes compound symmetry. In compound symmetry, the assumption is that the correlation between data points within individual animals is constant. ARIMA better models the situation where the correlation decreases with increasing 'distance' between the points, for example measurements taken at time point 1 and time point 2 are more similar than measurements taken at time point 1 and time point 5.

For each measurement point, separate GEE models were used to evaluate the effect of the explanatory variables dose and time, and feed and time, on the two outcome measures, median intra-day pH and %t>pH4. Models assessing the effect of drug dose were run for each type of feed and measurement point, and conversely models assessing the effect of feed were run for each dose of drug and measurement point. Median intra-day pH and %tpH>4 for day 1 to day 5 were included for each model. Baseline (day 0) median intra-day pH or %tpH>4 was included as a covariate in each model. Within each model day 1 was compared day 5. A Bonferroni correction was applied to these comparisons resulting in a P value of <0.0016 being considered significant. For all other comparisons significance was set at P<0.05. Graphs were created using GraphPad Prism (www.graphpad.com/scientific-software/prism).

6.4.6 Animal ethics

The study was performed under an ethics permit from the New South Wales Department of Primary Industries (TRIM 14/2710 (7)).
6.5 Results

The majority of the data were normally distributed. From the 578 data points there were 22 (3.8%) missing values. Figures 6.2 and 6.3 show the effect of dose and diet on mean %t>pH4 and mean median intra-day pH, respectively.

6.5.1 Effect of diet and location

There was no difference between mean %t>pH4, over days 1 to 5 combined, at measurement point 1 on the HG/LF compared to the HAY diet on either dose. However, at measurement point 2 the mean %t>pH4, over days 1 to 5 combined, was higher on the HG/LF diet than the HAY diet when the horses were on a 4 mg/kg dose at (69% vs. 22% respectively, p<0.001) and at the 1 mg/kg dose (49% vs. 16% respectively, p<0.001).

There was no difference in mean median intra-day pH, over days 1 to 5 combined, on the HG/LF diet compared to the HAY diet when the horses were on a 4 mg/kg dose at measurement point 1. However, mean median intra-day pH, over days 1 to 5 combined, was higher on the HG/LF diet compared to the HAY diet when on a 4 mg/kg dose at measurement point 2 (4.9 vs. 2.9 respectively, p<0.001). There was no difference between mean median intra-day pH, over days 1 to 5 combined, on the HG/LF compared to the HAY diet when the horses were on a 1 mg/kg dose at either measurement point 1 or 2.

6.5.2 Effect of Dose and location

The mean %t>pH4, over days 1 to 5 combined, was higher on the 4 mg/kg dose compared to the 1 mg/kg dose at both measurement points when horses were on the HG/LF diet (88% vs. 57% respectively at measurement point 1, and 66% vs. 28% respectively at measurement point 2, p<0.001). The mean %t>pH4, over days 1 to 5 combined, at point 2 was also higher on the 4 mg/kg dose vs. the 1 mg/kg dose on the HAY diet (19% vs. 4% respectively, p=0.03). There was no difference in mean %t>pH4, over days 1 to 5 combined, on the 4 mg/kg dose compared to the 1 mg/kg dose at measurement point 1 on the HAY diet.

There were no differences in mean median intra-day pH, over days 1 to 5 combined, on the 4 mg/kg dose compared to the 1 mg/kg dose on the HAY diet at either location (measurement point 1 or point 2). The mean median intra-day pH, over days 1 to 5 combined, was higher on the 4 mg/kg dose compared to the 1 mg/kg dose when horses
were on the HG/LF diet at measurement point 1 (6.4 vs. 4.3 respectively, p<0.001) and also at measurement point 2 (4.8 vs. 2.5 respectively, p<0.001).

6.5.3 Effect of time

The mean %t>pH4 was higher on day 5 compared to day 1 at measurement point 2 on both doses on the HG/LF diet (38% vs. 12% respectively on 1 mg/kg and 75% vs. 49% respectively on 4 mg/kg, p=0.001). When comparing day 5 of treatment to day 1 of treatment, mean median intra-day pH was higher on day 5 at measurement point 2 on both doses and in both diets (1.9 on day 1 vs. 3.3 on day 5 on the HAY diet at a 4 mg/kg dose, 3.8 on day 1 vs. 5.3 on day 5 on the HG/LF diet at a 4 mg/kg dose and 1.4 on day 1 vs. 3.0 on day 5 at a 1mg/kg dose on the HG/LF diet and 3.6 vs. 5.2 on the HG/LF diet at a 4 mg/kg dose on the HG/LF diet, p<0.001).

There was a positive correlation between %t>pH4 at measurement point 1 and at measurement point 2 (r_s=0.86, p=0.01), but no correlation between median intra-day pH at the two points (r_s=0.06, p=0.77), as shown in figure 6.4.

6.6 Discussion

The results of the present study were surprising as the overall efficacy of omeprazole in raising ventral gastric pH was less than previously reported. Further, the present study suggested that both dose and diet may play a role in the efficacy of omeprazole in the horse. As such, the use of singular dosing recommendations that encompass all horse types and usages, with their associated differences in management, as traditionally recommended [1,17], may not be appropriate. Alternatively, tailored dosing regimens that take into account diet may allow more effective usage of omeprazole in clinical practice with lower doses appropriate under conditions similar to the HG/LF diet in the present study and higher doses required for horses on ad libitum roughage-based diets.

The results of the current study showed a lower than expected overall efficacy of omeprazole. As such the authors consider it appropriate to review the previous studies on which this expectation was based. An early study investigating the pharmacodynamics of omeprazole in the horse reported the administration of 1.4 mg/kg of enteric-coated pellets in gelatine capsules via nasogastric tube for 6 days [4]. Using a model that involved collection of gastric fluid from an indwelling gastric cannula, gastric acid output and acidity
were determined. On day 7 basal gastric acidity and acid output were determined between 24-25 hours post-omeprazole administration and pentagastrin stimulated acidity and acid output determined between 26-27 hours. On day 7 at 24-25 hours post dose, basal acid output (mmol H+/15 min) was decreased by 90%, while gastric acidity (mmol H+/L) was decreased by 66%. Pentagastrin stimulated gastric acid output at 26-27 hours was reduced by 72% and mean pH (3.2 ± 1.5) was significantly higher than control values (1.7 ± 0.7) [4].

Another study reported the efficacy of an omeprazole paste at 4 mg/kg and 5 mg/kg PO once daily [3]. Basal and pentagastrin stimulated gastric acid output and pH was evaluated 21-24 hours after 15 doses in thirteen horses. The results of the study suggest that at 4 mg/kg PO once daily profound acid suppression was achieved with basal and pentagastrin stimulated gastric acid output reduced by 83% and 90%, respectively, on day 15 (hours 21-24) [3]. Similarly, on day 15 gastric pH was 5.5 ± 0.47 and 4.32 ± 0.62 (basal and pentagastrin stimulated, respectively) at 21-24 hours post-omeprazole administration. No differences were present between the 4.0 mg/kg and 5.0 mg/kg dose [3].

In contrast, a more recent study by Merritt et al measured intra-gastric pH over 24 hour periods using pH probes inserted retrograde into the indwelling gastric cannula in six horses fed ad libitum hay supplemented with a small grain meal twice daily [11]. GastroGard®, which was compared to three compounded formulations of omeprazole, only achieved a pH exceeding 4 for only 14 and 11 hours on days 2 and 7 of treatment, respectively. This is equivalent to a %t>4 of 58% and 46%, respectively. The time pH exceeds 4 was used as the determinant of efficacy as this is widely accepted in human medicine as the primary determinant of symptomatic control and healing for the treatment of gastroesophageal reflux disease (GERD), a condition the has many similarities with squamous ulceration in the horse [11].

The results of the present study are more consistent with the Merritt et al [11] study and probably reflect the methodology used to measure gastric acidity. The earlier studies fasted the horses for 18 – 24 hours prior to the collection of gastric fluid [3,4]. This intervention is potentially relevant as fasting increases the bioavailability, as determined by area-under-the-curve (AUC), of buffered paste omeprazole formulations by approximately 200-300% when compared with values from fed horses [3,109]. The primary determinant of efficacy of omeprazole in humans [102] and dogs [110] is AUC, and, as such, conditions that increase AUC may increase the efficacy of omeprazole. Conversely, conditions, such
as the *ad libitum* diet used in the present study and that reported by Merritt *et al* [11] may decrease the bioavailability, and thus efficacy of omeprazole. However, it should be noted that direct comparison between the findings of the present study and that of Merritt *et al* is difficult as the experimental methodology had subtle, but potentially significant differences. These include the location of the gastric cannula which was truly ventral in the Merritt *et al* study [11] and located below the lesser curvature in the present study, and the depth of insertion of the probes, reported as “5 cm into the gastric lumen” in the Merritt *et al* study [11] and approximately 10-20 mm and 60-70 mm for measurement point 1 and 2, respectively, in the present study.

Further uncertainty was apparent in the earlier studies since it is not known whether fasting itself will affect gastric acid production from the parietal cell, or if the pentagastrin stimulation model used in these studies induced gastric acid output equivalent to feeding. It is therefore possible that the experimental design of these earlier studies fluid may have inadvertently and incorrectly concluded a higher efficacy of omeprazole than that which is achieved when it is administered to fed horses.

An important outcome from the current study was that omeprazole at 4 mg/kg PO once daily in the high grain/low fibre diet was highly efficacious in the suppression of gastric acidity with a predicted %t>pH4 exceeding 80% from day 1 onwards. These findings are consistent with clinical studies in Thoroughbred racehorses on the same diet where resolution of ESGD, as evaluated by endoscopy, is reported to be 70-80% with 28 days of omeprazole therapy at 4 mg/kg PO once daily [5–7,10,50]. Interestingly, a recent study has reported non-inferiority of 1 mg/kg, compared with 4 mg/kg, PO once daily in the treatment of clinical ESGD in a Thoroughbred racehorse population fed a similar diet [80], which suggested that lower doses may be equally efficacious clinically under specific conditions [8]. Although the non-inferiority study used an enteric-coated formulation of omeprazole that differed from the buffered formulation evaluated in the current study, two recent studies have found no-significant difference in bioavailability, as measured by AUC, between the two formulations [69,104]. As such it is unlikely that the efficacy of the low dose was attributable to the formulation used. Instead, it suggests that the %tpH>4 achieved at 1 mg/kg PO once daily is adequate for ESGD healing to occur, at least under the conditions studied.

In contrast to the magnitude of acid suppression observed in the HG/LF, relatively poor acid suppression was observed in the HAY diet (predicted day 5 %tpH>4 was
approximately 40% and 30% for measurement points 1 and 2, respectively, on 4 mg/kg PO once daily, and 30% and <10% for measurement points 1 and 2, respectively, on 1 mg/kg PO once daily). Similarly, relatively low levels of suppression were observed at measurement point 2, when compared with measurement point 1. Although the exact location of the tip of the probe, and thus measurement point 2, was not known it can reasonably be expected that it is likely to be buried in the ingesta. Accordingly it is likely that the ingesta had an effect on the regional pH, and would likely have muted the magnitude of changes in measured pH as well as potentially affecting the dynamics of such changes.

The authors propose that the low magnitude of suppression observed with the HAY diet suggest that dose reduction of omeprazole below 4 mg/kg PO once daily is not advisable for the treatment of EGUS in patients on an ad libitum hay/roughage based diet. Further, the results of the present study suggest that current dosing regimens may not be appropriate for use in horses on ad libitum hay/roughage based diets. Instead the use of higher doses, twice daily dosing or alternative PPI’s, which may results in higher magnitudes of acid suppression appears to warrant investigation. This may be particularly relevant for the treatment of EGGD as the outcome reported with omeprazole monotherapy in recent clinical studies using current dosing regimens is poor [8–10].

The cumulative effect of treatment wherein the magnitude of acid suppression observed on day 5 was greater than that for day 1 has not, to the authors' knowledge, been previously reported for the horse. In interpreting the cumulative effect, it should be noted that the effect was not consistently observed under all conditions and that a statistically significant effect could only be demonstrated at measurement point 2 under specific conditions. In contrast, visual examination of the data suggests that such an effect is not be present at measurement point 1, which likely more accurately reflects conditions at the level of the mucosa. As such the clinical significance of the cumulative effect demonstrated is questionable.

It has been previously suggested that such a cumulative effect is not present in the horse [11]. In humans, the efficacy of proton pump inhibitors (PPI’s) is cumulative as their short half-life (90 minutes in humans) means that not all pumps will be active during the short period when PPI’s are present in the blood [66], so not all pumps will be inactivated each day. This effect is likely to be more pronounced in the horse given its even shorter half-life of approximately 30 minutes [74,100]. Alternatively it has been suggested in man
that repeated administration of omeprazole may result in impaired clearance due to inhibition of the cytochrome p-450 elimination pathway by either omeprazole or a sulphone metabolite [79]. A third potential cause that has been proposed is that a self-protective effect, wherein subsequent doses of omeprazole are protected by the increase in intra-gastric pH that occurs following administration of previous doses, may be present [79].

Another interesting finding of the present study was the differences observed between the two measurement points of gastric pH. For example, there were data points where the median intra-day pH at measurement point 1 exceeded 7 while median intra-day pH at measurement point 2 was <2 (Figure 6.4). Similarly, although the correlation between the probes for %t>pH4 was classified as good, examination of the raw data suggests that a wide range of correlations are present. For example, some values for %t>pH4 at measurement point 1 of 100% corresponded with values of 0% at measurement point 2. This finding was surprising, given that the measurement points were located only 5 cm apart from each other. A similar effect has been reported in humans, both pre and post-prandial, when intra-gastric pH is measured in multiple locations [111]. Regional pocketing of acidity has been described in humans [112] and appears to warrant further investigation in the horse, especially when monitoring response to treatment with acid suppressive agents as this was when the differences between the two measurement points were most obvious (data not shown). The %t>pH4 and median intra-day pH for measurement point 1 were consistently higher than measurement point 2, although the clinical significance of this is not known. It has been suggested in human medicine that a single pH electrode does not adequately detect regional differences in the stomach and the measurement at multiple probe locations may improve diagnostic accuracy [111]. Based on these recommendations and the findings of the present study, the authors propose that future studies measuring intra-gastric acidity in the horse should measure intra-gastric pH at multiple locations to improve diagnostic accuracy and allow more thorough evaluation of potential therapeutic agents, and in particular acid suppressive therapy.

One potential weakness of the current study was that the horses in the HG/LF diet were not specifically fasted. However, it has recently been reported that many stabled horses finish an evening meal before midnight [113] and this was observed by the authors of the present study wherein the entire meal in the HG/LF group was consistently consumed within 4 hours. This effectively resulted in a fast of approximately 10 hours prior to the morning administration of the medication. Further, the authors believe that the
study protocol reflects the real world usage of the medications and thus the findings are more applicable to the clinical setting. Another potential weakness is that the presence of the PEG tube and gastropexy may have altered gastric motility. Analysis of pharmacokinetic data collected simultaneously (see chapter 7) on days 1 and 5 reveals similar time to maximal concentration values as previously reported in fed horses, suggesting that this effect may be less likely, although it cannot be completely discounted. Further, a similar model has previously been reported and considered reliable for the measurement of intra-gastric acidity in the horse [11].

6.7 Conclusions

The findings of the present study suggest that both dose and diet affect the response to omeprazole in the horse. Relatively high levels of acid suppression were consistently observed in the HG/LF diet. Together with the previously reported efficacy of low dose (1 mg/kg PO once daily) omeprazole in clinical patients the findings suggest that lower doses of omeprazole may be suitable under conditions which replicate the feeding of the HG/LF diet in the present study. In contrast, the response in the *ad libitum* hay based diet was consistently poor. These findings suggest that dose reduction is not appropriate in a population receiving a roughage based diet. Instead higher dose, twice daily or alternative PPI therapies appear to warrant further investigation.
Figure 6.1 - A gastroscopic image showing final placement of the pH probe with two measurement points 5 cm apart. The first measurement point is located approximately 10–20 mm from the surface of the mucosa. The second measurement point sits freely within the ingesta/fluid contents of the ventral stomach.
Figure 6.2 – The effect of dose (1 mg/kg PO once daily and 4 mg/kg PO once daily) on mean %$\text{pH} > 4$ at two measurement points in six horses on either a high grain/low fibre diet or an *ad libitum* hay only diet.
Figure 6.3 - The effect of dose (1 mg/kg PO once daily and 4 mg/kg PO once daily) on mean median intra-day pH at two measurement points in six horses on either a high grain/low fibre diet or an *ad libitum* hay only diet.
Figure 6.4 – Scatter plots demonstrating the relationship between daily measurements at two different points (measurement point 1 and measurement point 2) 5 cm apart in the ventral stomach of 6 horses.

$r_s=0.86$, $(p=0.01)$

$r_s=0.06$, $(p=0.77)$
Chapter 7 – The effect of dose and diet on the pharmacokinetics of omeprazole in the horse.

This chapter consists of a paper submitted to the Journal Veterinary Pharmacology and Therapeutics as:


7.1 Prelude

As discussed in the prelude to chapter 6 the findings of the earlier studies in this thesis were primarily focused on the influence of diet and formulation on the pharmacokinetics of omeprazole in the horse. The findings of chapter 4 suggested that the impact of formulation was minimal. However, bioequivalence between commercially available formulations could not be determined. As such the authors considered it appropriate to select GastroGard® as the formulation for evaluation in the final study since it has historically been the most widely used formulation globally.

Although diet could not be shown to be a significant influence in the studies reported in chapter 2 or 3, the authors felt that the raw data and previously published literature suggested that an effect may be present. As such further investigation was warranted and two diets, representing difference ends of the clinical spectrum, were included for evaluation. Lastly, the effect of dose has been poorly described with no reports of direct comparisons between doses published.

Considering this the objective of the study was to further investigate;

- The effect of two diets, representing different ends of the clinical spectrum on the pharmacokinetics of GastroGard® in the horse; and
- The effect of two doses on the pharmacokinetics of GastroGard® in the horse.

7.2 Abstract

This study aimed to investigate the effect of diet and dose on the pharmacokinetics of omeprazole in the horse. Six horses received two doses (1 mg/kg and 4 mg/kg) of omeprazole orally once daily for 5 days. Each dose was evaluated during feeding either a high grain/low fiber (HG/LF) diet and an ad libitum hay (HAY) diet in a four-way cross over
design. Plasma samples were collected for pharmacokinetic analysis on days 1 and 5. Plasma omeprazole concentrations were determined by ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS).

In horses being fed the HG/LF diet, on day one the Area-Under-the-Curve (AUC) and maximal plasma concentration (C\text{max}) were higher on the 4 mg/kg dose than on the 1 mg/kg dose. The AUC was higher on day five compared to day one with the 4 mg/kg dose on the HG/LF diet. On days one and five the AUC and C\text{max} were higher in horses being fed the HG/LF diet and receiving the 4 mg/kg dose than in horses being fed the HAY diet and receiving the 1 mg/kg dose. These findings suggest that both dose and diet may affect pharmacokinetic variables of omeprazole in the horse.

7.3 Introduction

Omeprazole is widely used in the horse for the treatment of equine gastric ulcer syndrome (EGUS). Yet to date the factors that affect the pharmacokinetics of omeprazole in the horse have been relatively poorly described. In humans the effect of cumulative dosing, which results in an increased bioavailability over time, is well documented [79], yet conflicting evidence exists whether such an effect is present in the horse. Studies on enteric coated granules demonstrated a cumulative effect to be present in one study with bioavailability increasing from 6% on day 1 to 13% on day 5 [4], although a different study reported a bioavailability of 12% and 14% on days 1 and 5, respectively [78]. Likewise, no cumulative effect of dosing was found between days 1 and 14 in a study evaluating the pharmacokinetics of a buffered formulation of omeprazole [3].

Further, the effects of feeding regimen and diet on omeprazole bioavailability in the horse are yet to be fully elucidated. A single study reporting the pharmacokinetics of a buffered formulation of omeprazole suggested a potentially significant impact of feeding wherein the area-under-the-curve (AUC) in fasted animals was approximately 300% greater than in fed animals [3]. In contrast, more recent studies found no significant effect of feeding on the bioavailability of an enteric-coated [100,109] or a buffered [109] formulation, although a trend of increased bioavailability with feeding was noted, particularly for the buffered formulation. It was therefore apparent that further studies into the impact of feeding on bioavailability were warranted.
One concern when comparing the pharmacokinetics of omeprazole in the horse in early studies was the relatively poor sensitivity of the analytical methods used, as evidenced by the failure to detect serum levels of omeprazole in some animals despite apparent acid suppression [4,78]. One study evaluating the administration of enteric-coated pellets in gelatine capsules at 0.7 mg/kg PO once daily found that the AUC could only be determined for 5/8 animals [78]. A second, similar study evaluating 1.4 mg/kg of enteric-coated pellets in gelatine capsules reported similar results with bioavailability of omeprazole only calculable for 5/8 horses studied [4]. Poor sensitivity of analytical methodologies, combined with a low and variable bioavailability, could reduce the validity of early studies to determine factors affecting the pharmacokinetics of omeprazole in the horse.

The objective of the present study was to further investigate the factors that affect the pharmacokinetics of omeprazole in the horse. Specifically; the effect of diet, dose and repeated administration over a 5 day period.

7.4 Material and Methods

GastroGard® (Merial, Duluth, GA, USA) was chosen as the study formulation as it has been the predominant formulation available globally for the past 15 years. It has been extensively evaluated in clinical studies that have documented its efficacy in the treatment of squamous gastric ulceration at a dose of 4 mg/kg PO once daily [5–7,50,94,95]. It has also been evaluated at a prophylactic for squamous ulceration with its efficacy at a dose of 1 mg/kg PO once daily documented [114–116].

7.4.1 Animals and animal ethics

Six healthy, adult Thoroughbred horses (aged 5-14 years, 480-565 kg bodyweight (BW)) consisting of three male castrates and three females were used. Each horse was instrumented with a percutaneous gastrotomy tube (PEG), as previously described [107], allowing for the concurrent measurement of intra-gastric pH as part of separate studies. Horses were housed in 16 m² stables and bedded on wood shavings throughout the study. Horses were fed ad libitum rye grass-lucerne hay mix that was supplemented with a commercial feed pellet (Equine Mare and Foal®, Vella Stock Feeds, Plumpton, NSW, Australia) as required to maintain body condition throughout the study period unless otherwise dictated by the diet under investigation. Water was available ad libitum at all
times. The study was performed under an ethics permit from the New South Wales Department of Primary Industries (TRIM 14/2710 (7)).

7.4.2 Group allocation

Horses were assigned to treatment pairs with one pair studied each week in a four-way cross over design. Two diets were evaluated; a hay only diet (HAY) or a high grain/low fibre (HG/LF) diet. The HG/LF diet consisted of 5 kg (1% BW per day for a 500 kg horse) grain (40% protein pellets (Equine Mare and Foal®, Vella Stock Feeds, Plumpton, NSW, Australia) and 60% sweet feed (Robank Non-Oat Custom Mix®, Robank, Ebenezer, NSW, Australia)) and 5 kg (1% BW per day for a 500 kg horse) hay, divided equally into two meals fed at 10 am and 6 pm to represent typical dietary management of a racehorse in training [80]. The HAY diet consisted of ad libitum access to rye grass-lucerne hay. Horses were adapted to the diets over a period of at least 1 week prior to the commencement of data collection. The remaining four horses rested and, where appropriate, transitioned between test diets. A minimum 2 week wash-out period was allowed between studies.

7.4.3 Administration of medication

Two doses of omeprazole were evaluated; 1 mg/kg PO once daily and 4 mg/kg PO once daily. The two doses were chosen as they are the registered GastroGard® doses for EGUS prevention (1 mg/kg) and treatment (4 mg/kg). To ensure accurate dosing each horse was weighed on electronic scales (Accuweigh Equestrian Scales, Accuweigh, Willeton, WA, Australia) on day -1. The omeprazole was administered using the graduated dose markings on the syringe, rounding up to the nearest 50 pound (22.7 kg) dose. Omeprazole was administered at 8 am (2 hours prior to feeding in the HG/LF diet) for a 5 day period from day 1 – 5.

7.4.4 Sample collection

An IV catheter was placed on the morning of sample collection (days 1 and 5) approximately 1 hour prior to the administration of the omeprazole to allow collection of the blood samples. A pre-administration blood sample was collected, the omeprazole administered and further blood samples collected from the catheter at T= 15, 30, 45, 60, 75, 90, 105 min and 2, 2.5, 3, 4, 6, 8 and 10 hours. Ten mL of blood was collected on each occasion with 4 mL transferred to a lithium heparin tube (Vacutainer®, BD, North
The samples were separated by centrifugation at 3,500 g for 10 min immediately following collection and the plasma frozen (-20 °C) within 30 min of collection.

7.4.5 Sample extraction and determination of plasma omeprazole concentration

Prior to analysis, plasma samples were allowed to thaw at room temperature and a protein precipitation extraction was performed; 100 µL of the sample was added to a 1.5 mL Eppendorf tube and 200 µL of a methanol: acetone mix at a ratio of 80:20 was added. The solution was then mixed by vortexing for 15 seconds, followed by centrifugation of the mixture at 15,000 g for 10 min. The supernatant was collected and the plasma omeprazole concentration determined by ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS) analysis as recently described [100].

The magnitude of intra-run variability was determined by calculating the coefficient of variation of the values for the replicates of the calibration curve samples determined at the beginning, middle and end of each run. Inter-run variability was determined by calculating the coefficient of variation between runs for each calibration point using average values within each run. Accuracy of the analysis was determined by calculating the coefficient of determination for the calibration curve using Microsoft Excel®’s (Microsoft, Redmont, WA, USA) graphing function.

7.4.6 Pharmacokinetic evaluation

The pharmacokinetic calculations were carried out by non-compartmental assessment of the data using an open source pharmacokinetic program (PK Solver, China Pharmaceutical University, Ninqing, China) [96]. Maximum plasma concentration (C_{max}) in plasma and the time required to reach C_{max} (T_{max}) was directly calculated from the data by the software program. The elimination rate constant (lambda-z) was estimated by log-linear regression of concentrations observed during the linear phase of elimination, using at least three data points automatically selected by the program and confirmed by visual examination of the plotted data. The area under the concentration vs. time curve (AUC_{0-∞}) was calculated using the linear trapezoidal rule.

7.4.7 Data analysis

Data were analysed using a computer software program (SPSS). Data were assessed for normality visually and using the Kolmogorov-Smirnov test with Dallal-
Wilkinson-Lille for P values. The data were non-parametric so were log transformed to allow parametric analysis. A repeated measures ANOVA with Tukey’s post-test was performed to compare the $AUC_{0-\infty}$, $C_{\text{max}}$, $T_{\text{max}}$ and half-life in each feed/dose variation (1 mg/kg on the HAY diet, 1 mg/kg on the HG/LF diet, 4 mg/kg on the HAY diet, 4 mg/kg on the HG/LF diet) on days 1 and 5. Paired $t$-tests were used to compare day 1 of treatment with day 5 of treatment for each feed/dose variation. Significance was set at $P \leq 0.05$. Summary statistics are expressed as mean ($\pm$ SD) or geometric mean with corresponding 95% confidence intervals (95%CI). Inter- and intra-run variability’s are reported as median [inter-quartile range: IQR].

7.5 Results

All horses successfully completed the study and no adverse events were noted. The actual dose of omeprazole given ranged from 1.01-1.012 mg/kg in the 1 mg/kg group and from 4.00-4.12 mg/kg in the 4 mg/kg group. A complete data set for all horses at all times was available. All sample concentrations fell within the standard curve. Median intra-run variability for omeprazole concentration was 10.9% [IQR: 6.4 – 33.6 %]. Median inter-run variability was 13.7% [IQR: 13.4 – 14.6 %]. The median coefficient of determination ($R^2$) for the calibration curve was >0.99 for all runs.

Figure 7.1 shows scatter plots for $AUC_{0-\infty}$, $C_{\text{max}}$, $T_{\text{max}}$ and half-life for each of the feed/dose variations. Table 7.1 shows mean and 95% confidence $AUC_{0-\infty}$, $C_{\text{max}}$, $T_{\text{max}}$ and half-life for each of the feed/dose variations.

Effect of dose

On day one there was an increase in the $AUC_{0-\infty}$ on the 4 mg/kg dose compared to the 1 mg/kg dose on the HG/LF diet (7.63 (95%CI: 2.63-22.12) $\mu$g min/mL vs. 1.16 (95%CI: 0.44-3.04) $\mu$g min/mL, $p=0.039$). On day five there was a trend towards the $AUC_{0-\infty}$ being higher on the 4 mg/kg dose when compared to the 1 mg/kg dose on the HG/LF diet (14.36 (95%CI: 4.84-42.59) $\mu$g min/mL vs. 2.01 (95%CI: 0.90-4.48) $\mu$g min/mL, $p=0.052$). There was no difference in the $AUC_{0-\infty}$ on the 1 mg/kg dose compared to the 4 mg/kg on the HAY diet on day 1 (0.87 (95%CI: 0.51-1.48) $\mu$g min/mL vs. 4.50 (95%CI: 0.95-21.48) $\mu$g min/mL, $p=0.14$) or day 5 (0.94 (95%CI: 0.41-2.13) $\mu$g min/mL vs. 4.37 (95%CI: 0.89-21.59) $\mu$g min/mL, $p=0.25$).
On day one the $C_{max}$ was higher on the 4 mg/kg dose than on the 1 mg/kg dose on the HG/LF diet (0.077 (95%CI: 0.021-0.283) µg/mL vs. 0.012 (95%CI: 0.004-0.033) µg/mL, $p=0.048$). One day five there was a trend towards the $C_{max}$ being higher on the 4 mg/kg dose than on the 1 mg/kg dose on the HG/LF diet (0.134 (95%CI: 0.035-0.506) µg/mL vs. 0.022 (95%CI: 0.010-0.051) µg/mL, $p=0.089$). There was no difference in the $C_{max}$ on the 1 mg/kg dose compared to the 4 mg/kg dose on the HAY diet on day 1 (0.010 (95%CI: 0.001-0.017) vs. 0.060 (95%CI: 0.011-0.340), $p=0.18$) or day 5 (0.011 (95%CI: 0.000-0.030) vs. 0.024 (95%CI: 0.003-0.182), $p=0.78$).

There was no difference in the $T_{max}$ on the 1 mg/kg dose compared to the 4 mg/kg dose on the HG/LF diet on day 1 (37.4 (95%CI: 18.5-75.4) minutes vs. 62.1 (95%CI: 33.1-116.4) minutes, $p=0.57$) or day 5 (45.9 (95%CI: 32.1-65.6) minutes vs. 43.1 (95%CI: 20.3-91.6) minutes, $p=0.99$). There was no difference in the $T_{max}$ on the 1 mg/kg dose compared to the 4 mg/kg on the HAY diet on day 1 (28.6 (95%CI: 14.4-56.9) minutes vs. 38.6 (95%CI: 28.3-52.5), $p=0.59$) or day 5 (37.4 (95%CI: 25.2-55.6) minutes vs. 43.3 (95%CI: 13.7-136.8) $p=0.99$).

There was no difference in the half-life on the 1 mg/kg dose compared to the 4 mg/kg dose on the HG/LF diet on day 1 (50.6 (95%CI 34.9-73.5) minutes vs. 69.8 (95%CI 40.2-121.1) minutes, $p=0.57$) or day 5 (50.2 (95%CI 34.2-73.7) minutes vs. 54.3 (95%CI 37.2-79.4) minutes, $p=0.99$). There was no difference in the half-life on the 1 mg/kg dose compared to the 4 mg/kg on the HAY diet on day 1 (42.9 (95%CI 22.4-82.1) minutes vs. 60.3 (95%CI 46.1-78.9) minutes, $p=0.59$) or day 5 (42.4 (95%CI 25.1-71.6) minutes vs. 83.2 (95%CI 42.6-162.4) minutes, $p=0.99$).

Effect of diet

There was no difference in the AUC$_{0-\infty}$ on the HAY diet compared to the HG/LF diet on the 1 mg/kg dose on day one (0.87 (95%CI: 0.51-1.48) µg min/mL vs. 1.16 (95%CI: 0.44-3.04) µg min/mL, $p=0.14$) nor on day five (0.93 (95%CI: 0.44-3.04) µg min/mL vs. 2.01 (95%CI: 0.90-4.48) µg min/mL, $p=0.25$). There was no difference in the AUC$_{0-\infty}$ on the HAY diet compared to the HG/LF diet on the 4 mg/kg dose on day one (4.50 (95%CI: 0.94-21.48) µg min/mL vs. 7.63 (95%CI: 2.63-22.12) µg.min/mL, $p=0.89$) nor on day five (4.37 (95%CI: 0.89-21.59) µg min/mL vs. 14.36 (95%CI: 4.84-42.59) µg.min/mL, $p=0.17$)

There was no difference in the $C_{max}$ on the HAY diet compared to the HG/LF diet on the 1 mg/kg dose on day one (0.010 (95%CI: 0.006-0.017) µg/mL vs. 0.012 (95%CI:
There was no difference in the C\textsubscript{max} on the HAY diet compared to the HG/LF diet on the 4 mg/kg dose on day one (0.060 (95%CI: 0.011-0.342) µg/mL vs. 0.077 (95%CI: 0.021-0.283) µg/mL, p=0.99) nor on day five (0.024 (95%CI: 0.003-0.182) µg/mL vs. 0.134 (95%CI: 0.035-0.506) µg/mL, p=0.28).

There was no difference in the T\textsubscript{max} on the HAY diet compared to the HG/LF diet on the 1 mg/kg dose on day one (28.6 (95%CI: 14.4-56.9) minutes vs. 37.4 (95%CI: 25.2-55.6) minutes, p=0.92) nor on day five (37.4 (95%CI: 26.2-55.6) minutes vs. 45.9 (95%CI: 32.1-65.6) minutes, p=0.84). There was no difference in the T\textsubscript{max} on the HAY diet compared to the HG/LF diet on the 4 mg/kg dose on day one (38.6 (95%CI: 28.3-52.5) minutes vs. 62.1 (95%CI: 33.1-116.4) minutes, p=0.32) nor on day five (43.3 (95%CI: 13.7-136.8) minutes vs. 43.1 (95%CI: 20.3-91.6) minutes, p=0.999).

There were no differences in the half-life on the HAY diet compared to the HG/LF diet on the 1 mg/kg dose on day one (42.9 (95%CI: 22.4-82.1) minutes vs. 50.6 (95%CI: 34.9-73.5) minutes, p=0.91) nor on day five (42.4 (95%CI: 25.2-71.6) minutes vs. 50.2 (95%CI: 34.2-73.7) minutes, p=0.95). There was no difference in the half-life on the HAY diet compared to the HG/LF diet on the 4 mg/kg dose on day one (60.3 (95%CI: 46.1-78.9) minutes vs. 69.8 (95%CI: 40.2-121.1) minutes, p=0.91) nor on day five (83.2 (95%CI 42.3-162.4) minutes vs. 54.3 (95%CI 37.2-79.4), p=0.37).

\textit{Combined effect of dose and diet}

On day one, the AUC was higher in horses receiving the 4 mg/kg dose on the HG/LF diet compared to those receiving the 1 mg/kg dose on the HAY diet (7.63 (95%CI: 2.63-22.12) µg min/mL vs. 0.87 (95%CI: 0.51-1.48) µg min/mL, p=0.036). This effect was also present on day 5 (14.36 (95%CI: 4.84-42.59) µg min/mL vs. 0.93 (95%CI: 0.41-2.13) µg min/mL, p=0.004). There were no other significant dose/diet interactions of the AUC on days one and five.

On day one the C\textsubscript{max} was higher in horses receiving the 4 mg/kg dose on the HG/LF diet compared to those receiving the 1 mg/kg dose on the HAY diet (0.077 (95%CI: 0.021-0.283) µg/mL vs. 0.010 (95%CI: 0.006-0.017) µg/mL, p=0.034). This effect was also present on day five (0.134 (95%CI: 0.035-0.506) µg/mL vs. 0.011 (95%CI: 0.004-0.030) µg/mL, p=0.02). There were no other significant diet/dose interactions of C\textsubscript{max} on days one
and five. There were no significant diet/dose interactions of $T_{\text{max}}$ and half-life on days one and five.

**Effect of time**

The AUC$_{0-\infty}$ was higher on day 5 compared to day 1 when horses were receiving the 4 mg/kg dose on the HG/LF diet (14.4 (95%CI: 4.8-42.6) µg min/mL vs. 7.6 (95%CI: 2.6-22.1) µg min/mL ($p=0.046$)). There were no further differences between the half-life, $T_{\text{max}}$, $C_{\text{max}}$ or AUC$_{0-\infty}$ on day 1 vs. those on day 5 for any of the feed and dose variations.

**7.6 Discussion**

To the authors' knowledge the relative pharmacokinetics of different doses of omeprazole has not been previously reported in the horse. Further, the impact of diet has been poorly investigated. The findings of the present study suggest that an effect of dose and, to a lesser extent, diet may be present on some pharmacokinetic variables of omeprazole in the horse. Mean AUC$_{0-\infty}$ and $C_{\text{max}}$ values for 4 mg/kg were consistently higher than for 1 mg/kg with the effect significant on day 1 for both AUC$_{0-\infty}$ and $C_{\text{max}}$. Likewise, mean AUC$_{0-\infty}$ and $C_{\text{max}}$ values for the HG/LF diet were consistently higher than values for the HAY diet, although the effect was not significant at any time point. However, when the effect of diet and dose were considered together a significant effect was present on day 5 with the HG/LF diet at 4 mg/kg having a higher AUC$_{0-\infty}$ and $C_{\text{max}}$ than the HAY diet at 1 mg/kg suggesting that both diet and dose may influence both AUC$_{0-\infty}$ and $C_{\text{max}}$. In contrast, no effect of diet or dose was present on half-life or $T_{\text{max}}$ suggesting that these variables are not affected by diet or dose. Both AUC$_{0-\infty}$ and $C_{\text{max}}$ were consistently approximately 5 – 7 times higher with the 4 mg/kg dose when compared with the 1 mg/kg dose. The magnitude of this increase is greater than the 4-fold increase in dose and suggests that dose linear pharmacokinetics may not be present in the horse. Further studies are needed to define this effect, but until further documented the authors consider that the extrapolation across doses as previously reported [69] to be inappropriate.

The inability to demonstrate a consistent effect of diet may be related to a number of factors including the magnitude of any such effect being relatively small, or due to a lower than adequate power to detect such an effect in the present study. The small number of animals and the wide degree of variability in the data, as evidenced by the wide 95% confidence intervals, suggests that low power may be relevant and warrants consideration in the interpretation of the results. The clinical relevance of these findings is
unclear as the relationship between pharmacokinetics and pharmacodynamics of omeprazole in the horse has not been described to date. However, in humans [102] and dogs [110] the primary determinant of omeprazole efficacy is AUC, and, as such, conditions that increase AUC may increase the efficacy of omeprazole.

A cumulative effect of dosing was present under conditions that favored omeprazole absorption, namely the HG/LF diet, and the higher (4 mg/kg) dose. The inability to demonstrate a consistent cumulative effect is consistent with previous reports in the horse [3,78] but contrasts to findings in man where AUC increases with repeated administration of 20 mg once daily [79]. In man it has been suggested that repeated administration of omeprazole may result in impaired clearance due to inhibition of the cytochrome p-450 elimination pathway by either omeprazole or a sulphone metabolite [79]. Alternatively, it has also been proposed that a self-protective effect, wherein subsequent doses of omeprazole are protected by the increase in intra-gastric pH that occurs following administration of previous doses, may be present [79]. Either effect may be favoured by higher doses and conditions that enhance absorption with either increased accumulation of inhibitory metabolites, or greater efficacy in intra-gastric acid suppression, theoretically plausible. Further work to elucidate the relationship between the pharmacokinetics and pharmacodynamics, and the inter-play between dose and diet, of omeprazole in the horse would be advantageous in elucidating both the relationship between pharmacokinetics and pharmacodynamics, and the relative impact of the proposed mechanisms for the cumulative effect observed at the higher dose with the HG/LF diet.

One potential weakness of the study is that the horses in the high grain/low fibre diet were not specifically fasted. However, it has recently been reported that many stabled horses finish an evening meal before midnight [113], an effect observed by the authors of the present study wherein the entire meal in the high grain/low fibre group was consistently consumed within 4 hours. This effectively resulted in a fast of approximately 10 hours prior to the morning administration of the medication. Further, the HG/LF diet was chosen as it is typical of the diet of Australian Thoroughbred racehorses [80,117] and as such the authors propose that it represents the clinical scenario more accurately.

Another potential weakness is that the presence of the PEG tube and gastropexy may have altered gastric motility. The use of PEG instrumented horses allowed for the concurrent measurement of intra-gastric pH as part of separate studies investigated the pharmacodynamics of omeprazole in the horse (chapter 6). A potential effect of the PEG
tube and gastropexy via alterations in gastric motility and emptying on the pharmacokinetics reported in this study cannot be completely discounted. However the author's consider it unlikely as the reported values for $T_{\text{max}}$, the parameter most likely to be affected by alterations in motility, were similar to previously reported values using the same formulation [3,104].

7.7 Conclusions

In conclusion the findings of the present study suggest that both dose and diet may affect some pharmacokinetic variables of omeprazole in the horse. Further, a cumulative effect of repeated administration was present under certain conditions. Further studies to elucidate the relationship between the pharmacokinetics and pharmacodynamics, and the inter-play between dose and diet, of omeprazole in the horse would be advantageous. Such studies may benefit from reducing the number of variables, such as diet, dose and formulation, investigated on each occasion. Further, higher doses may warrant investigation.
Table 7.1 - Mean and 95% confidence intervals for Area-Under-the-Curve ($AUC_{0-\infty}$), $C_{\text{max}}$, $T_{\text{max}}$ and half-life on days 1 and day 5 for the two doses (1 mg/kg and 4 mg/kg orally once daily) and two diets (high grain/low fiber (HG/LF) and *ad libitum* hay (HAY)). Groups with the same superscript letter are statistically different from each other (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>1 mg/kg</th>
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<th>4 mg/kg</th>
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<tbody>
<tr>
<td></td>
<td>HG/LF</td>
<td>HAY</td>
<td>HG/LF</td>
<td>HAY</td>
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<tr>
<td></td>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 1</td>
<td>Day 5</td>
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<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>AUC$_{0-\infty}$</strong> ($\mu g\cdot min/ml$)</td>
<td>1.16$^{a}$ 0.44-3.04</td>
<td>2.01 0.90-4.48</td>
<td>0.87$^{c}$ 0.51-1.48</td>
<td>0.94$^{d}$ 0.41-2.13</td>
</tr>
<tr>
<td><strong>C$_{\text{max}}$</strong> ($\mu g/ml$)</td>
<td>0.012$^{b}$ 0.004-0.033</td>
<td>0.022 0.010-0.051</td>
<td>0.010$^{a}$ 0.001-0.017</td>
<td>0.011$^{f}$ 0.000-0.030</td>
</tr>
<tr>
<td><strong>$T_{\text{max}}$</strong> (min)</td>
<td>37.4 18.5-75.4</td>
<td>45.9 32.1-65.6</td>
<td>28.6 14.4-56.9</td>
<td>37.4 25.2-55.6</td>
</tr>
<tr>
<td><strong>Half-life (min)</strong></td>
<td>50.6 34.9-73.5</td>
<td>50.2 34.2-73.7</td>
<td>42.9 22.4-82.1</td>
<td>42.4 25.1-71.6</td>
</tr>
</tbody>
</table>

Note: The same superscript letters indicate statistically similar groups (p<0.05).
Figure 7.1 – Scatter plots of Area-Under-the-Curve (AUC$_{0-\infty}$), $C_{\text{max}}$, $T_{\text{max}}$ and half-life on days 1 and day 5 for the two doses (1 mg/kg and 4 mg/kg orally once daily) and two diets (high grain/low fiber (HG/LF) and ad libitum hay (HAY)). Groups with the same superscript letter are statistically different from each other ($p<0.05$).
Chapter 8 – Pharmacokinetic/pharmacodynamic modelling of omeprazole in the horse

8.1 Introduction

In dogs [110,118] and humans [102] the gastric anti-secretory effect of omeprazole is related to the area under the plasma concentration curve. The objective of this study was to preliminarily investigate whether such an effect was present in the horse utilising pharmacokinetic and pharmacodynamic data collected concurrently in study 5.

8.2 Materials and Methods

8.2.1 Animals, group allocations, sample collection and analysis

Horses were allocated and managed as outlined in chapter 6. Concurrent pharmacodynamic and pharmacokinetic measurements were performed on days 1, 3 and 5. Pharmacodynamic measurements were performed as outlined in chapter 6. Collection and analysis of plasma samples was performed as outlined in chapter 7. Pharmacokinetic analysis was performed as outlined in chapter 7.

8.2.2 Data analysis

Data for mean intra-day pH, median intra-day pH and the percentage time pH was above 4 (%tpH>4) for both measurement points (measurement point 1 and measurement point 2) and the three days (days 1, 3 and 5) were screened graphically for correlations against the key pharmacokinetic variables (C_max and AUC_0-∞). Spearman’s correlation coefficients were used, with coefficients <0.4 regarded as poor, 0.4–0.7 moderate and >0.7 good [108].

8.3 Results

All horses successfully completed the study and no adverse events were noted. Median intra-run variability for omeprazole concentration was 10.9% [IQR: 6.4 – 33.6 %]. Median inter-run variability was 13.7% [IQR: 13.4 – 14.6 %]. The median coefficient of determination (R^2) for the calibration curve was >0.99 for all runs.
Due to technical errors with intra-gastric pH measurement, data was not available for 2 horses on day 1 (both HG/LF diet and 1 mg/kg dose), one horse on day 3 (HG/LF diet and 4 mg/kg dose) and one horse on day 5 (HAY diet and 4 mg/kg dose). Plasma omeprazole concentrations peaked at 0.001 μg/mL (the limit of quantification) in one horse on days 3 and 5 (HG/LF diet and 1 mg/kg dose) and did not allow calculation of AUC$_{0-\infty}$. Where either the intra-gastric pH measurements or the key pharmacokinetic variables were not available the entire data for that time point was excluded. This resulted in a total of 66 complete data sets available for comparison.

Figures 8.1 – 8.6 show key pharmacodynamic variables (mean intra-day pH, median intra-day pH and %tpH>4) plotted against $C_{\text{max}}$. Figures 8.7 – 8.12 show key pharmacodynamic variables (mean intra-day pH, median intra-day pH and %tpH>4) plotted against AUC$_{0-\infty}$. All correlation coefficients were classified as poor (<0.4).

8.4 Discussion

In the present study no correlation between either of the pharmacokinetic parameters (AUC and $C_{\text{max}}$) and pharmacodynamic parameters (mean intra-day pH, median intra-day pH and %tpH>4) could be demonstrated. This is in contrast to studies in dogs [110,118] and humans [102] where the gastric anti-secretory effect is related to the area under the plasma concentration curve.

The reasons for the failure to observe such an effect is unclear. The number of animals in the present study was small although studies in dogs have used as little as three dogs to accurately model pharmacokinetic/pharmacodynamic data [110]. Alternatively the overall poor low $C_{\text{max}}$ and AUC values observed in the present project may explain the lack of correlations. Examination of the scatter plots in figures 8.1 – 8.12 reveals a clustering of data points at the low end of both scales with a wide range of corresponding mean intra-day pH, median intra-day pH and %tpH>4 values. Lastly, the impact of meal feeding on baseline pH measurements in the HG/LF diet may have distorted the data. Equally plausible is the impact of continuous feeding in the hay diet. Human and dog studies are typically conducted in fasted patients and the effect of a large bolus of food in the stomach, especially a stomach as large as the horses, is not known. Further modelling of subsets of the data focusing on higher doses and conditions that results in better absorption (HG/LF diet or fasted animals) may result in more reliable modelling and warrants consideration.
Figure 8.1 - Mean intra-day pH vs. $C_{\text{max}}$ at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.
Figure 8.2 - Mean intra-day pH vs. C_{max} at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[ y = 4.2286x + 2.5899 \]

\[ R^2 = 0.2371 \]
Figure 8.3 - Median intra-day pH vs. $C_{\text{max}}$ at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

$y = 6.915x + 3.9224$

$R^2 = 0.2077$
Figure 8.4 - Median intra-day pH vs. $C_{\text{max}}$ at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[ y = 5.3975x + 2.4605 \]

$R^2 = 0.2481$
Figure 8.5 - %tpH>4 vs. $C_{\text{max}}$ at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

$y = 118.58x + 44.749$

$R^2 = 0.1926$
Figure 8.6 - %tpH>4 vs. C_{max} at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[ y = 108.68x + 18.372 \]

\[ R^2 = 0.2563 \]
Figure 8.7 - Mean intra-day pH vs. AUC$_{0-\infty}$ at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[ y = 0.0717x + 3.9202 \]

\[ R^2 = 0.2095 \]
Figure 8.8 - Mean intra-day pH vs. AUC$_{0-\infty}$ at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

$y = 0.0623x + 2.4848$

$R^2 = 0.3858$
Figure 8.9 - Median intra-day pH vs. \( \text{AUC}_{0,\infty} \) at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[
y = 0.0813x + 3.9224
\]

\[
R^2 = 0.2174
\]
Figure 8.10 - Median intra-day pH vs. AUC$_{0-\infty}$ at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[
y = 0.0786x + 2.3351
\]

R$^2 = 0.3959$
Figure 8.11 - %tpH>4 vs. AUC$_{0-\infty}$ at measurement point 1. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

\[ y = 1.4126x + 44.647 \]
\[ R^2 = 0.2074 \]
Figure 8.12 - %tpH>4 vs. AUC$_{0-\infty}$ at measurement point 2. All data points from days 1 and 5 from a four-way cross over study design evaluating the effects of 2 doses (1 mg/kg and 4 mg/kg/day PO once daily) and 2 diets (an ad libitum hay diet and a high grain/low fibre diet) are included.

$y = -0.1624x + 27.433$

$R^2 = 0.0046$
Chapter 9 – General Discussion

Equine Gastric Ulcer Syndrome (EGUS) is arguably the most prevalent disease of the athletic horse with a prevalence of up to 100% reported in competing horses in some studies [19]. Up to 93% of Thoroughbred horses in training [19] and competing endurance horses [28] have ESGD and a prevalence of ESGD exceeding 50% has been reported in a wide range of other horse use types [29–32]. Similarly, a prevalence of EGGD exceeding 50% has been reported in a wide range of horse use types studies [8,31,33,34,38]. Omeprazole is considered the treatment of choice [1], supported by clinical studies that document its efficacy over other treatment options [50,68]. However, the typical response rate reported in clinical studies assessing omeprazole at 4 mg/kg PO once daily for 28 days, the most commonly used dose globally, is only approximately 70 – 80% for ESGD [5–7,49,50]. Further, in studies performed by the authors in the lead up to this thesis it was demonstrated that the healing rate decreases to 25% for EGGD lesions under similar conditions [8–10]. To date the failure of approximately 20 – 30% of horses with ESGD and approximately 75% of horses with EGGD to heal has been poorly investigated.

Considering this the aim of the present project was to investigate the factors that affect the pharmacokinetics and pharmacodynamics of omeprazole in the horse with the ultimate aim of elucidating potential explanations for the variability in treatment response described above. To achieve this, the project was conducted in three parts;

- Part 1: This formed a preliminary evaluation of the factors that affect the pharmacokinetics of omeprazole in the horse. The effects of feeding and formulation were evaluated in three studies;

- Part 2: This involved the development and validation of a modified technique of intra-gastric pH measurement in the horse;

- Part 3: This involved integration of the findings from part 1 with the model developed in part 2 for a detailed assessment of the pharmacokinetics and pharmacodynamics of omeprazole over a 5 day period. Further, an attempt was made to correlate the pharmacokinetic data with the pharmacodynamic data.
Part 1

Study 1

The initial study in the project, as reported in chapter 2, evaluated the pharmacokinetics of an enteric coated formulation and a plain formulation of omeprazole. At the time of study execution little was published on the pharmacokinetics of enteric coated omeprazole with the data that was available incomplete due to the inability to detect plasma concentrations in several animals in each study [4,78]. Further, the effect of feeding on the pharmacokinetics of enteric coated omeprazole had not previously been reported, in contrast to buffered omeprazole where the impact appears to be marked [3], nor had the pharmacokinetics of plain, unprotected omeprazole been determined.

The findings of the study were an overall bioavailability of a single dose for the enteric coated omeprazole in the fasted horse of 22% [100]. This is greater than previously reported under similar conditions with previous reports ranging from 6% [4] to 12% [78]. The most likely explanation for the greater bioavailability observed is that the analytical technique used (UHPLC) in the present study is more sensitive than the technique used in the earlier reports (HPLC). This is evidenced in part by the current technique’s ability to detect plasma concentrations in all animals and under all conditions studied in the current project (although AUC could not be calculated at 2 time points due to the very low concentrations reported as detailed in chapter 7). It is particularly relevant in the measurement of AUC as the cumulative effect of the tail on the right hand side of the AUC plot can dramatically impact on the overall AUC recorded. Alternative explanations for the greater bioavailability observed include the specific formulation evaluated and dose used (4 mg/kg PO vs. 0.7 mg/kg PO [78] and 1.4 mg/kg PO [4]). These two factors were further evaluated in studies number 3 (reported in chapter 4) and number 5 (reported in chapter 7), respectively.

The findings of the study supported the recommendation that omeprazole be protected against gastric acidity [11] as the bioavailability of the plain, unprotected omeprazole (10%) was approximately half of the enteric coated formulation (22%). It should be noted that chapter 2 it is reported that a generic formulation of plain, unprotected omeprazole had recently been released onto the European market (Peptizole®). At the time of writing and based on the information available at the time this information was considered accurate as this was the manner in which the formulation was marketed. Recently the manufacturers of the aforementioned product have clarified that
their product is indeed buffered, primarily as a response to the ECEIM consensus statement [1] that clearly outlines the need to distinguish between different formulation types of omeprazole. Considering the low bioavailability found in study 1 and the absence of commercially available plain formulations, further evaluation of plain, unprotected formulations was not deemed justified.

The third, potentially significant finding of study 1 was the observation of wide range in bioavailability between individual animals. Three of 12 horses appeared to absorb omeprazole poorly, regardless of the formulation or feeding status studied. These findings are largely understated in the early studies outlining the pharmacokinetics of omeprazole in the horse, although a 1999 publication by Daurio [3] hinted at such an effect. In that study, plasma AUC’s with a standard deviation up to 80% of the mean value were reported although the presentation of the data as mean ± S.E. meant that the magnitude of the variation was less obvious on initial review. The finding that 25% of horses appeared to absorb omeprazole poorly was interesting as it appears to correlate well with the reported 20 – 30% treatment failure rate for ESGD under clinical conditions, leading the authors to speculate that individual variation in the absorption of omeprazole may be a key contributory factor in the observed therapeutic failure rates.

The final finding of study 1 was that the bioavailability of enteric coated omeprazole did not appear to be significantly affected by feeding. This is in direct contrast to a previous reported for a buffered formulation of omeprazole wherein feeding reduced the bioavailability, as measured by AUC, to approximately 1/3 of fed values [3]. This finding provided the justification for study 2 of the current project.

Study 2

As discussed above the primary justification for study 2 was the finding that the impact of feeding on an enteric coated formulation was less than previously reported for a buffered formulation of omeprazole suggesting that there may be an interaction between formulations and feeding. Such an interaction would be potentially relevant in formulations dosing recommendations.

The findings of the study were consistent with previous studies with feeding resulting in a reduction of median AUC to 57% and 49% of fasted values in the enteric coated and buffered formulation, respectively. Although a statistically significant effect of feeding was not found in the present study, likely due to the wide variation in the data,
these findings, in conjunction with previous reports [3,100], formed the justification for inclusion of diet as a key variable of interest in part 3 of the project.

Study 3

The third study evaluated the relative pharmacokinetics of 5 different formulations of omeprazole. At the time of study execution there was no published data on the relative bioavailability of different formulations of omeprazole beyond that reported in chapter 2. Interest in the bioavailability of different formulations is particularly pertinent to the Australian market as both enteric coated and buffered formulations of omeprazole are available commercially. The manufacturers of enteric coated omeprazole claim higher bioavailability (c.f. buffered formulations) yet, to the authors’ knowledge, no published data exists to support this claim. Between study execution and publication an independent report refuted this claim reporting no difference between the relative bioavailability of Gastrozol® at 1 mg/kg PO once daily and GastroGard® at 4 mg/kg PO once daily [69]. Further, no difference in endoscopic healing was observed between the two formulations [69] consistent with a study performed by the current authors wherein doses of an enteric coated formulation as low as 1 mg/kg PO once daily were shown to be as efficacious as 4 mg/kg PO once daily [8]. If the different formulations of omeprazole were equivalent then the implications are significant as the potential to use lower doses would result in significant cost savings to horse owners, potentially increasing the number of animals treated. As outlined in chapter 4, GastroGard® was chosen as the reference formulation as it is the best recognised internationally.

The findings of the study were that no differences were present between the enteric coated formulations, and one of the buffered formulations and GastroGard® (Merial, USA). These findings were consistent with the independent report [69] aforementioned. A longer \( T_{\text{max}} \) and lower \( C_{\text{max}} \) were present between the fourth test formulation (Abgard®) and GastroGard® but the significance of these clinically is questionable based on previous published reports of the formulation’s efficacy in clinical patients [9,119]. Bioequivalence could not be demonstrated for any of the formulas against GastroGard®. This is likely in part due to the small number of animals included in the study but examination of the raw data suggests that small, differences may be present although the clinical significance of these is unknown.

The lack of demonstrable bioequivalence was the primary justification for inclusion of GastroGard® as the formulation investigated in the part 3 of the project in that although
the other formulations were not demonstrated to be different they equally were not demonstrated to be the same. As such, GastroGard® was chosen as it is formulation best recognised internationally and it is generally considered the reference formulation.

The lack of a significant difference between the formulations was also the justification for inclusion of the low (1mg/kg PO once daily) dose in the final study. As discussed above two studies have found no difference in endoscopic outcome between 1 mg/kg PO once daily and 4 mg/kg PO once daily [8,69] raising the question whether lower doses may be equally efficacious for treatment.

Part 2

As discussed in chapter 6; several key limitations are present in the models that have been previously used to investigate intra-gastric pH in the horse. Of the previously used models the indwelling pH probe model fitted retrograde through a gastric cannula is arguably the most relevant to clinical conditions as it allows recording of intra-gastric pH under clinically relevant dietary conditions. The main downside of the previously described model [11] is the need for general anaesthesia and advanced surgical skill. The technique described in chapter 5 results in a comparable model for intra-gastric pH measurement while removing these disadvantages. The procedure was well tolerated and repeatable measurements of intra-gastric pH were recorded over the 5 day study period. At the time of writing the 6 instrumented horses used in study 5 have been maintained with indwelling PEG tubes for periods of 18 – 24 months and continue to be used in on-going studies. The primary disadvantage of the current model is that the fixed location of the probe only allows measurement at a specific anatomical location in the stomach. Given that pH within the horses’ stomach is stratified [40], it is important to note that measurement of intra-gastric pH in the present model is reflective in ventral gastric pH and may not be reflective of acidity in other regions of the stomach. Further, although well tolerated, placement of the PEG tubes is invasive and a period of convalescence is needed before animals can be used experimentally. Lastly, it is possible that gastropexy formed by the technique may alter gastric motility, although based on the concurrent pharmacokinetic measurements performed in the current studies this does not appear likely.

An interesting effect of meal feeding that has not previously been described was observed in this study and has reliably been observed in day 0 baseline recordings throughout the subsequent studies. The effect observed was a rapid rise in intra-gastric pH following meal feeding in the HG/LG diet as shown in figure 5.7. A similar effect has
been reported in humans where, due to the buffering effects of food, intra-gastric pH is highest after a meal [111]. It is also likely that the ingestion of saliva plays a significant role in the increase in pH observed. In the present studies the effect was most consistently observed following the morning meal with a less consistent effect present in the evening meal. The authors proposes that the reason behind the difference in effect at the two meals likely relates to the different inter-meal durations with the morning meal occurring 16 hours after the evening meal and the evening meal 8 hours subsequent to the morning meal. As such it is reasonable to expect that the stomach was emptier, and or the horse was hungrier and as a result ate faster, at the time of morning meal feeding which would exacerbated the buffering effect of a bolus of food. As shown in table 5.1 the %tpH>4 was approximately 0% in the hay only diet and approximately 10% in the HG/LF diet, although the clinical significance of this relatively modest effect is unclear.

Part 3

As discussed in part 1; based on the findings of studies 1 – 3, the key variables that were considered for inclusion in the final study were dose and diet. Since formulation was considered less important, GastroGard® was chosen as the test formulation as it is the international reference standard.

As discussed in chapter 6; the findings of the study suggest that the use of uniform dosing recommendations that encompass all horse types may not be appropriate. Although toxicity, dose related or otherwise, associated with the administration of omeprazole has not been reported in the horse, in most countries the per day cost of omeprazole therapy at 4 mg/kg PO once daily remains high, despite the expiration of the GastroGard® patent in early 2014. As such, the ability to use low dose therapy would be financially advantageous and would potentially allow the treatment of a greater number of animals due to greater affordability of the medication.

In considering the data presented in chapter 6 the relative validity of the findings to ESGD and EGGD healing conditions warrants discussion. The location of the pH probe within the ventral stomach provides a reliable indicator of pH in the region and the day 0 baseline recordings consistently reported a median intra-day pH of approximately 2 which is comparable to previous reports [40]. The authors consider that extrapolation of the ventral gastric pH data to ESGD healing conditions may be appropriate in the high grain/low fibre diet model, as intermittent fasting has been shown to disrupt the normal stratification of intra-gastric pH [120]. Equally the authors consider that interpretation of
ESGD healing conditions using ventral gastric pH measurements is likely not appropriate under *ad libitum* hay/roughage conditions as stratification of pH from 3 in the ventral stomach to 7 at the level of cardia is normally present under such conditions [40]. As such the pH recorded at the ventral measurement points is unlikely to be indicative of pH at the level of the squamous mucosa under such conditions.

**Implications of the Present Study**

**Formulation**

The findings of the present project reinforce previous recommendations that omeprazole requires some form of protection from intra-gastric acidity to prevent its degradation during transit through the stomach [11]. However, they suggest that the method of protection used, either enteric coating or buffering, is less important than the presence of a protective mechanism *per se* at least in the fasted horse. Whether the mechanism of protection has an impact of absorption in horses receiving *ad libitum* hay/roughage based diets is less clear. The results of study 1 and 2 suggest that an interaction between formulation and feeding may be present wherein the impact of feeding on reducing bioavailability may be less pronounced in enteric coated formulations than buffered formulations. No statistically significant effect could be demonstrated in the present project therefore the authors consider that caution should be observed in extrapolating the findings of the present study relating to fed conditions between formulation types. Further work into the specific impact of feeding on the pharmacokinetics and pharmacodynamics of enteric coated omeprazole are required before conclusion as to its efficacy under fed conditions can be drawn.

**Dose**

Based on previous reports on the apparent efficacy of low dose (1 mg/kg PO once daily of an enteric coated formulation (Gastrozol®) omeprazole therapy in the treatment of ESGD [8,69] and the findings of the present project it appears that a recommendation of 1 mg/kg PO once daily for the treatment of horses receiving high grain/low fibre diets is justified. Based on the findings of study 3, it does not appear that the choice of a buffered or enteric coated formulation is likely to impact upon this recommendation as it is likely that horses are effectively fasted prior to administration of omeprazole each day under such conditions. The efficacy of GastroGard® at 2 mg/kg PO once daily in the prevention of ESGD in Thoroughbred racehorses [68] and the registration of buffered omeprazole at 1
mg/kg (UlcerGard®, Merial, Duluth, GA, USA) appears to support this recommendation. It has been demonstrated that the risk of ESGD in racehorses with once daily administration of an oral buffered omeprazole formulation (GastroGard®) at 2 mg/kg PO once daily does not differ from the risk with administration at 4 mg/kg PO once daily.

Further, to the authors’ knowledge it has not been demonstrated that treatment requires a greater duration of acid suppression than prevention. Given that the mechanism of treatment and prevention are identical (i.e. indirect via suppression of acid production) it is reasonable to consider that the efficacy of buffered formulations at 1 mg/kg PO once daily as a prophylactic may also translate to efficacy in treatment at the same dose. Further, differences for time to healing and ESGD scores were not different between horses administered a range of dosing regimens (1.5 mg/kg PO twice daily, 3 mg/kg PO once daily, 3 mg/kg PO twice daily and 6 mg/kg PO once daily) [95]. However, it should be noted that a dose response was present in study 5 and as such it is emphasised that several assumptions and extrapolations are made in the above statement. Clinical studies evaluating 1 mg/kg PO once daily of buffered omeprazole for the treatment of ESGD are required before a firm recommendation can be made.

In contrast, based on the findings of the present study the use of reduced doses for horses receiving ad libitum hay or roughage based diets does not appear justified. Although both 1 mg/kg and 4 mg/kg PO once daily were equally effective in raising %tpH>4 and median pH, as shown in figures 6.2 and 6.3 respectively, it appears that the neither dose resulted in adequate suppression of acid production to allow healing of lesions in the ventral or glandular region of the stomach under such conditions. Predicted day 5 %tpH>4 was < 40% which, based on reports in other species is inadequate for healing to occur. In humans good healing rates for GERD are achieved when the %tpH>4 exceeds 66% [88]. It warrants note that %tpH>3 is used as a benchmark for glandular ulcer healing in other species and may more accurately predict the efficacy of current dosing regimens for glandular healing than %tpH>4 with a %t>pH >3 of greater than 66% required in humans [88]. Unfortunately due to limitations in the software of the reporting program determination of %tpH>3 was not possible. However, the duration and magnitude of acid suppression required for healing of either ESGD or EGGD has not been documented, and the authors proposes that %tpH>4 represents a more conservative benchmark for the investigation of acid suppressive therapy until such factors are documented. This statement has been supported in a recent [85] review.
It is tempting to speculate that the relatively poor ability of omeprazole to raise ventral gastric pH under certain conditions is a potential explanation for the reported low healing rate of EGUSD with omeprazole monotherapy [8–10]. However, more detailed interpretation of the published data suggests that such a conclusion may not be appropriate. The conditions studied in 2 of the papers cited [8,9] included administration 1–4 hours prior to feeding in a clinical scenario comparable to the model evaluated in study number 5. Under such conditions the administration of 4 mg/kg PO once daily would be expected to result in marked suppression of acid production as shown in the high grain/low fibre diet in figure 6.2 where the %tpH>4 exceeded 80% from days 1-5. This finding draws into question the recommendation that acid suppressive therapy form a cornerstone of the treatment of EGUSD. Further studies are needed to document the healing rate of EGUSD under conditions where marked acid suppression is expected. Further studies evaluating 4 mg/kg PO once daily in horses receiving high grain/low roughage diets are needed before acid suppressive therapy can be discounted as a pre-requisite for EGUSD treatment. Pharmacodynamic studies evaluating twice daily administration or higher doses are needed to determine the treatment regimen that could reasonably be expected to result in conditions conducive to EGUSD healing.

Feeding Recommendations

Current feeding recommendations for the management of EGUS do not discriminate between EGUS and EGUSD or between management during treatment and management during prevention. The most common recommendations include the provision of ad libitum roughage, a reduction in the grain content of the diet and supplementation with vegetable oil [1]. These recommendations are based primarily on risk factors identified for EGUS and are arguably less relevant to the management of EGUSD as dietary factors are yet to be consistently identified as risk factors for EGUSD [1].

In particular reduction of grain content is expected to be beneficial as increased starch/grain intake has been associated with an increased risk of EGUSD in animals working at various levels of intensity in a number of studies. A marked increase in ulceration has been observed when non–exercising animals were stabled and fed grain at 1% of BW, 1 hour before hay was fed [47]. Similarly, exceeding 2 g/kg BW of starch intake per day has been associated with an approximately two-fold increase in the likelihood of EGUS grade ≥ 2/5 [48]. In another study, all horses developed EGUS within 14 days of their removal from pasture, stabling (fed 6 kg concentrate feed/day) and entering a
simulated training regimen [64], although it warrants note that the effects in this latter study are likely multifactorial. The provision of vegetable oil is logical as both total gastric acid output and prostaglandin concentration has been shown to decrease in ponies with gastric cannulas fed 45 mL corn oil orally once daily by dose syringe [121].

However, the benefits of ad libitum roughage are less clear and conflicting evidence is present in the literature as to whether pasture turn out or ad libitum roughage reduces the risk of ESGD. No effect of quality of pasture, or time at pasture (stabled, stable and pasture, pastured) was shown on ESGD prevalence in a study of Thoroughbred racehorses in New Zealand [25]. Further, in a study evaluating the influence of a high fibre diet vs. an iso-energetic low fibre diet both the number and severity of ESGD lesions was greater in the high fibre diet group [122]. Together, these findings suggest that that the impact of forage feeding in the absence of other risk factor reduction may not be as great as previously believed [1].

Considering this and the impact of ad libitum hay on the efficacy of omeprazole demonstrated in the present study the dietary recommendation of ad libitum roughage warrants discussion. In the absence of pharmaceutical intervention the provision of ad libitum roughage is a small management change, one that may be advantageous and one that is logical to implement. However, during treatment with omeprazole the significantly greater magnitude and duration of acid suppression observed with fasting should be considered and balanced against the potential, but unproven, benefits of ad libitum roughage. Paradoxical to the common believe that ad libitum roughage is ideal, the authors believes that it may be advantageous to enforce a brief overnight fast prior to the administration of omeprazole in animals undergoing treatment. Based on the findings of the current studies the duration of that fast should not be less than 10 hours as it is not known whether shorter duration fasts result in a similar increase in omeprazole efficacy. Further investigation into the effect of shorter fasting periods warrants investigation and determination of the shortest possible fasting period that allows a trade-off between omeprazole efficacy and the provision of the maximal intra-day access to roughage would be advantageous. The application of such an approach may be particularly useful in the management of EGGD as the benefits of ad libitum roughage in reducing the risk of disease are less clear. As such the risk: benefit ratio of fasting shifts further in favour of fasting to enhance omeprazole efficacy. The development of dosing regimens or the use of alternative therapeutics that result in an adequate magnitude and duration of acid suppression under ad libitum roughage conditions would be ideal as it would represent a
“best of both worlds’ situation in which nutritional management and pharmaceutical efficacy are both optimised.

In addition to the enforcement of an overnight fast it is logical that feeding should coincide with peak plasma concentrations of omeprazole, or shortly thereafter to allow transportation of omeprazole in the parietal cell, as omeprazole works best when parietal cell activation coincides with high drug concentrations [87]. Based on the findings of the present studies; $T_{\text{max}}$ is $\leq 100$ minutes in the majority of animals suggesting that a delay of approximately $1\frac{1}{2} – 2$ hours between administration of the omeprazole and feeding is appropriate in the horse.

**Inter-individual variability**

As discussed in chapter 2, a wide range of inter-individual variability was observed in study 1 and this effect was present throughout the project. The plotted data in figures 8.2 – 8.5 further supports this statement with individual pharmacodynamic response highly variable regardless of the dose or diet studied. The observation that approximately 25% of animals fail to absorb omeprazole appears to roughly correlate with the pharmacodynamic data from study 5 and it is tempting to speculate that this finding potentially explains the failure of 20 – 30% of patients with ESGD to heal in clinical studies [5–7,49,50]. Further investigation into the pharmacodynamics of clinical non-responders appears justified and the potential use of alternative therapeutics, such as ranitidine [5,39,71,72], in such patients warrants consideration. The failure to address predisposing factors such as diet [46] and high intensity exercise [45] may be confounding factors.

**Future directions**

**Formulation**

The findings of the present project suggest that little difference exists between the commercially available formulations, at least in the fasted state. As discussed above further evaluation of the pharmacodynamics of enteric coated formulations appears warranted to definitively document their efficacy, although based on the findings of the present project the expected differences would likely be small.

Alternatively the investigation of different PPI’s appears justified, especially in the fed state. In humans a wide range of PPI’s are used with esomeprazole one of the better studied. The use of esomeprazole as the PPI of choice in humans has been supported by
two meta-analysis studies demonstrating its superiority over other PPI’s in treating clinical disease [123,124]. The use of esomeprazole has recently been described in the horse [90,125] and it appears to have promise as an alternative means of acid suppression that warrants further investigation.

**Dose**

As discussed above investigation of higher doses or alternative regimens appears warranted. Pilot investigations by the authors have suggested that 8 mg/kg PO once daily of GastroGard® results in rapid and profound (%tpH>4 exceeds 80% by day 1) acid suppression even in horses fed *ad libitum* hay. As discussed above, it is unclear whether increased acid suppression will result in an increase in the EGGD healing rate but further assessment of the pharmacodynamics, and ultimately clinical efficacy, of high dose therapy is logical.

Alternatively twice daily administration warrants discussion. No advantage of twice daily administration, over once daily administration, was observed in a study evaluating ESGD response rates over a range of doses (1.5 mg/kg PO twice daily, 3 mg/kg PO once daily, 3 mg/kg PO twice daily and 6 mg/kg PO once daily) [95]. In humans the predicted magnitude of acid suppression with omeprazole increases from 66% of maximal acid output with once daily administration to 80% with twice daily administration [66]. Considering that the impact of twice daily administration appears to be modest the authors consider that the investigation of higher doses warrants consideration first. Additionally the maintenance of a once daily administration is likely to improve compliance should higher dose regimens be demonstrated to be superior.

**Genetics**

The consistent effect of inter-individual variability is an interesting finding of the present project. The cause(s) of such an effect are not known. In humans, variation in the efficacy of omeprazole has been reported and is primarily related to mutations in the CYP2C19 gene and changes in the rate of elimination of omeprazole [98]. Whether the poor bioavailability observed in some horses in the present project reflects increased clearance of omeprazole, as observed in humans, is unclear but evaluation of the genetic profile of the horse, with specific relevance to the CYP2C19 gene, appears warranted. Alternatively factors that affect the rate of absorption may be important.
Pharmacokinetic/pharmacodynamic modelling

As discussed in chapter 8 no correlation between key pharmacokinetic parameters and key pharmacodynamic parameters was demonstrable although the reasons for this are unclear. If future studies into higher dose omeprazole support the pilot findings reported above and higher levels of suppression are observed with such doses then further investigations into pharmacokinetic/pharmacodynamic modelling may be warranted.
Bibliography


60. Murray, M.J. and Eichorn, E.S. (1996) Effects of intermittent feed deprivation, intermittent feed deprivation with ranitidine administration, and stall confinement with


Appendix 1 – Additional publications relevant to the thesis but not forming part of it


Sykes, B. W., Sykes, K. M. & Hallowell, G. D. Administration of once daily trimethoprim-sulphadimidine does not improve healing of glandular gastric ulceration in horses receiving