To the Graduate Council:

I am submitting herewith a thesis written by Rilla Evelyn Reese entitled: “Mixture of Volatile Fatty Acids: Effect on bioelectric properties of acid injury in the equine non-glandular stomach.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters of Science, with a major in Animal Science.

_____
Frank Andrews, Major Professor

We have read this thesis
and recommend its acceptance:

_____
Richard Heitmann

_____
Kelly Robbins

Accepted for the Council:

_____
Carolyn R. Hodges
Vice Provost and Dean of the
Graduate School

Mixture of Volatile Fatty Acids: Effect on bioelectric properties of acid injury in the equine non-glandular stomach.

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Rilla Evelyn Reese
May 2009

This thesis is dedicated to my parents, Marilla Reese and Rufus Reese, and the rest of my family. Thanks for always believing in me and encouraging me in everything that I do.

Acknowledgements

I am extremely grateful for this opportunity to continue my education and for this I would like to thank Dr. Frank M. Andrews and the Animal Science Department.

Dr. Frank Andrews, my major professor, gave me an amazing opportunity to learn from one of the best teachers, researchers and people. He has been more than a mentor, he has been a friend. Dr. Andrews has guided, encouraged and supported me through my journey of completing my Masters degree and for this I am extremely thankful.

Within the Animal Science Department I would like to thank Dr. Richard Heitmann and Dr. Kelly Robbins for their advice and for serving on my committee. I am also thankful for the help, support and guidance of Dr. Alan Mathew. I would like to thank Dr. Arnold Saxton for all of the help on the statistical analysis of my data and his patience and flexibility with me. I would like to thank Dr. Maria Prado and Dr. Henry Kattesh for the opportunity to learn different sides of academia.

I have had the opportunity to make some amazing friends along the way. I would like to thank Dr. Ferenc Tóth for his friendship and for explaining to me different aspects of veterinary medicine. I am extremely thankful for the friendship and technical assistance of Ms. Sarah Elliott. Within the Animal Science Department I have made many friends and I would like to thank them for listening to my trials and tribulations and

mainly for being a friend: Brian Campbell, Tabitha Cooper, David Roper, Erin Fitsgerald, Eddie Jarboe, and Linda Miller.

I would like to thank my family for there unwavering love, support and encouragement throughout graduate school.

I would also like to thank the Comparative Gastrointestinal Society for the funding that made this study possible.

Abstract

The purpose of this study was to expose viable non-glandular (NG) mucosa from recently euthanized horses to Normal Ringers solution (NRS) and VFA mixtures (sub-threshold VFA mixture and VFA mixtures containing high acetic acid, high butyric acid and high propionic acids in NRS), at different pHs (1.5, 4.0, 7.0) in an *in vitro* Ussing Chamber system and to measure their effects on tissue bioelectric properties. After tissue exposure to NRS or VFA mixtures in NRS, tissue was examined under light microscopy for histopathologic evidence of cell swelling and necrosis. Horses (n=11) were euthanazied and gastric tissue obtained. Tissues, except from one horse with severe ulcers, were then mounted in Ussing Chambers and NRS or VFA mixtures were added to the mucosal side of the chamber and NRS at pH 7.0 was added to the serosal side. The pH of the mucosal side was dropped at 30 minutes to pH 1.5, 4 or 7 and exposed for 330 minutes. Tissue short circuit current (Isc) and potential difference (PD) was measured and resistance and conductance calculated every 15 minutes throughout the study. At the conclusion of the study, tissues were removed from the Ussing Chamber and placed in 10% formalin and stained with hematoxylin and eosin for histopathologic examination. NG mucosa exposed to HCl alone (pH 4.0 and 1.5) caused an immediate significant decrease in

PD, followed by a decrease in Isc. NG mucosa when exposed to the sub-threshold VFA mixture caused no significant change in PD or Isc, when compared to NRS at the same pH. However, NG mucosa exposed to above-threshold VFA mixtures caused an immediate significant decrease in PD and Isc. Results suggest that a sub-threshold mixture of VFAs do not cause bioelectric changes in the equine NG mucosa other than that caused by HCl alone. However, if the concentrations of VFAs exceed the threshold level of propionic and butyric acids at pH <4.0, changes in the barrier function occured. This study confirms that when stomach VFA concentrations are at or below threshold, tissue barrier function is maintained; whereas when stomach VFA concentrations exceed threshold concentrations, as found with high grain diets, at pH ≤ 4.0, cause damage to the NG mucosa which may lead to the formation of ulcers.

Table of Contents

CHAPTER I. Review of the Literature 1
Volatile Fatty Acids 1
VFA Structure and Function 1
VFA Production and Metabolism 2
VFA Absorption 5
Equine and porcine stomachs: Comparative anatomy and physiology 6
Histology of the monogastric stomach 7
Physiology of gastric secretions 10
Gastric Ulcers in Horses 12
Anatomy 12
Diet 13
Restricted Feed Intake 14
Environmental Stresses 15
Helicobacter spp 17
Ussing Chamber System 17

CHAPTER II. Objectives 19

CHAPTER III. Materials and Methods 20
Animals and Treatments 20
Ussing Chamber Techniques 21
Preparation for Histopathologic Examination 23
Statistical Analysis 24

CHAPTER IV. Results 25
Animals and gastric tissue 25
VFA Mixes and control exposure to mucosa 25

CHAPTER V. Discussion 34
Animals and gastric tissue 34
VFA Mixes and control exposure to mucosa 34
Literature Cited 41
Appendix 48
Vita 86

List of Tables

Table I-1 Classification of VFAs abbreviated from Bugaut 1987[2] 1
Table III-1 Treatment solutions and the composition 22

Table A 1 Breakdown of horses by age 49
Table A 2 Breakdown of horses by sex 49
Table A 3 Breakdown of horses by reason for euthanasia 50
Table A 4 Breakdown of horses by Gastrophilus intestinais larvae present in the stomach 50
Table A 5 Breakdown of horses by gastric ulcer score 51
Table A 6. Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa 70
Table A 7 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa. 71
Table A 8Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa. 72
Table A 9 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa. 73
Table A 10 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa. 74
Table A 11 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa. 75
Table A 12 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short logresistance (R) in equine nonglandular mucosa. 76
Table A 13 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logresistance (R) in equine nonglandular mucosa. 77
Table A 14 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logresistance (R) in equine nonglandular mucosa. 78
Table A 15 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa. 79
Table A 16 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa. 80
Table A 17 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa. 81
Table A 18 Effect of time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa. 82
Table A 19 Effect of time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa. 83
Table A 20 Effect of time *pH at pH 1.5, 4.0 and 7.0 on logresistance (R) in equine nonglandular mucosa. 84
Table A 21 Effect of time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa. 85

List of Figures

Figure I.1 Flow chart of the summary of VFA production pathways in the rumen[3] 4
Figure I.2 Structure of gastric pit that opens into the gastric gland and the different cell types[15] 8

Figure A 1 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 52
Figure A 2 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses 52
Figure A 3 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 53
Figure A 4 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 53
Figure A 5 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 54
Figure A 6 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 54
Figure A 7 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 55
Figure A 8 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 55
Figure A 9 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 56
Figure A 10 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 56
Figure A 11 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 57
Figure A 12 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 57
Figure A 13 Time and pH interaction. Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses 58
Figure A 14 Time and pH interaction. Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 58
Figure A 15 Time and pH interaction. Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 59
Figure A 16 Time and pH interaction. Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 59
Figure A 17 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 60
Figure A 18 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 60

Figure A 19 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 61
Figure A 20 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 61
Figure A 21 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses. 62
Figure A 22 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses. 62
Figure A 23 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses. 63
Figure A 24 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses. 63
Figure A 25 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses. 64
Figure A 26 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses. 64
Figure A 27 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 65
Figure A 28 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 65
Figure A 29 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 66
Figure A 30 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 66
Figure A 31 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses. 67
Figure A 32 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 67
Figure A 33 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 68
Figure A 34 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 68
Figure A 35 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 69
Figure A 36 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses. 69

Nomenclature

µA	microamp
µm	micrometer
ml	milliliter
mmhos/cm²	millimhos per square centimeter
mM	millimolar
mv	millivolts
min	minute
ohms/cm²	ohms per square centimeter

Abbreviations

Ca	calcium
CO_2	carbon dioxide
CCK	cholecystokinin
Cl	chloride
G	conductance
HPO_4	dibasic sodium phosphate
R	electrical resistance
EGUS	equine gastric ulcer syndrome
GIP	gastric inhibitory peptide
GI	gastrointestinal
H^+	hydrogen ion
HCl	hydrochloric acid

LSD	least significant difference
Mg	magnesium
H_2PO_4	monobasic sodium phosphate
NRS	normal Ringers solution
K	potassium
KCl	potassium chloride
PD	potential difference
Isc	short circuit current
Na	sodium
VFAs	volatile fatty acids

CHAPTER I. Review of the Literature

Volatile Fatty Acids

VFA Structure and Function

Volatile fatty acids (VFAs) are one to seven carbon compounds and exist as branched or straight chains (Table 1-1). VFAs principally acetic, propionic and butyric and to a lesser extent valeric, isovaleric and isobutyric are produced along with small amounts of other organic compounds, such as methane, carbon dioxide, alcohol and lactate, through microbial fermentation within the gastrointestinal (GI) tract in people and animals[1].

Table I-1 Classification of VFAs abbreviated from Bugaut 1987[2]

VFA Structure	Trivial Name	Systematic Name	Shorthand Designation
CH_3COOH	Acetic	Methanoic	2:0
CH_3CH_2COOH	Propionic	Propanoic	3:0
$CH_3(CH_2)_2COOH$	Butyric	Butanoic	4:0
$(CH_3)_2CHCOOH$	Isobutyric	Isobutanoic	i 4:0
$CH_3(CH_2)_3COOH$	Valeric	Pentanoic	5:0
$(CH_3)_2CHCH_2COOH$	Isovaleric	Isopentanoic	i 5:0

VFAs are produced as microbial waste product during the fermentation process. Microbes make adenosine triphosphate (ATP) during fermentation, which is then utilized for the maintenance and growth. The VFAs are important because when absorbed supply 60% to 80% of the dietary energy to ruminants and horses[3,4]. The concentrations and proportions of VFAs produced in the GI tract differ depending on the age of the animal, components of the diet, time elapsed after feeding, and location in the GI tract[5]. A study completed in 1945 found acetic acid (67%), propionic acid (19%), and butyric acid (14%) in the alimentary canal of the sheep, ox, red deer, horse, pig and rabbit. Also, proportional concentrations in ruminants which included 40-75% for acetic acid, 15-40% for propionic acid and 10-20% for butyric acid [1,2].

VFA Production and Metabolism

Dietary carbohydrates such as cellulose, hemicellulose, starch and pectin are the main substrates that are fermented by bacteria. They are broken down to their hexoses and pentoses before being fermented to VFAs via pyruvate. Pentoses are converted to hexose and triose phosphate in the pentose cycle by transketolase and transalodolase reactions. Majority of carbohydrate metabolism proceeds using hexose which is metabolized to pyruvate in the Embdem-Meyerhof glycolytic pathway. Pyruvate is the precursor to acetic, propionic and butyric acids in most cases. Acetyl CoA is an intermediate when acetic and butyric acid

is fermented, whereas the formation of propionic acid occurs mainly via succinate pathway and to a lesser extent the pathway involving acrylate. The reactions can be summarized in the following equations and with the flow chart (Figure 1-1)[3]:

$$\text{Hexose} = 2\ \text{Pyruvate} + 4H$$

$$\text{Pyruvate} = \text{Acetate} + CO_2 + 2H$$

$$2\ \text{Pyruvate} = \text{Butyrate} + 2CO_2$$

$$\text{Pyruvate} + 4H = \text{Propionate} + H_2O$$

$$CO_2 + 8H = \text{Methane} + 2H_2O$$

Early studies have show measurable concentrations of VFAs within the stomach of non-ruminants[6-9] including the horse, pig, dog, rabbit and rat with highest concentrations in the most proximal portion of the stomach, where pH is moderate and supports the growth of acidophil bacteria[2]. While the production of VFAs is the highest in the cecum and large colon[1], there have been measurable concentrations found in the equine stomach[9,10].

In a study where horses were fed a high protein, high calcium diet (alfalfa hay/ grain) showed significantly higher stomach pH and VFA concentrations 2 to 5 hours after feeding when compared to horses fed a low protein, low calcium brome grass hay diet. VFA concentrations were

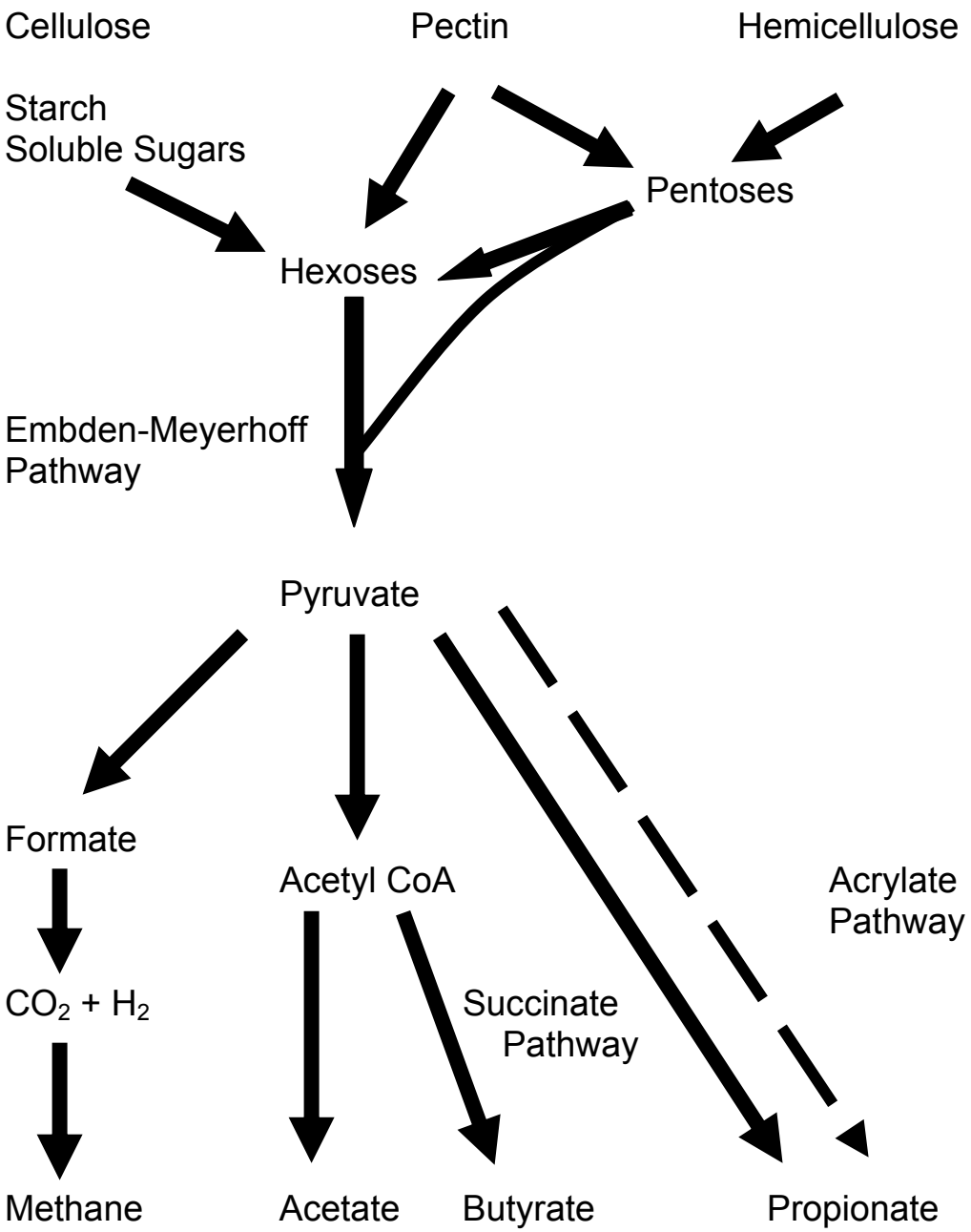

Figure I.1 Flow chart of the summary of VFA production pathways in the rumen[3]

the highest from 2 to 6 hours after feeding and decreased as the stomach emptied. On either diet, acetic acid concentration was the highest in the stomach when compared to other VFAs. The VFA concentrations ranged from: 16-20 mmol/L of acetic acid, 0.03-1.08 mmol/L of propionic acid, 0-1.6 mmol/L of butyric acid, and 0-6 mmol/L of D^-/L^+ lactate. Mean concentrations of isobutyric, valeric and isovaleric acid were low in gastric juice of those horses and significantly decreased during the first 4 hours after feeding with either diet[10].

VFA Absorption

During fermentation bacterial VFA concentrations increase and if allowed to build up suppress fermentation. Therefore, efficient removal of VFAs is essential to allow for the fermentation process to proceed. In ruminants, nearly all produced VFAs are absorbed through stratified squamous epithelium of the forestomach while 10-20% escape into the lower GI tract [4]. These VFAs are absorbed into the vascular system via simple diffusion and absorption is dependent on the rumen pH and intracellular metabolism[1]. The exact molecular mechanism of VFA absorption is not completely understood but is pH dependent[4]. The VFAs are weak acids with a pK of ≤ 4.8, and at a rumen pH of 5.8, most VFAs are dissociated or ionized. However, rumen VFA absorption increases when pH decreases, as VFAs become undissociated or free fatty acids, which are lipid soluble[1]. Because of the VFAs' increased lipid solubility,

they are able to passively diffuse through the cell membrane down a concentration gradient[2]. Once inside the cell each VFA has a different fate. Most acetic acid is absorbed unchanged, but a small amount is completely oxidized within the cell. Propionic acid is absorbed, with a small portion being converted to lactate within the cell. Butyric acid is metabolized to β–hydroxybutyrate, a ketone body, before absorption[4]. Each product is then absorbed into the vascular system[1].

Equine and porcine stomachs: Comparative anatomy and physiology

The equine and porcine stomachs are anatomically similar and are divided into three mechanically functional zones. The dorsal portion or the fundus receives and stores feed. The body or corpus mixes saliva and gastric juices with the food material for digestion. Finally, the antrum mechanically breaks down feed materials under high antral pressure and this zone regulates propulsion of feed through the pyloric sphincter [11].

Horses and pigs have a compound stomach consisting of non-glandular mucosa dorsally and glandular mucosa ventrally. The porcine stomach has a small portion of non-glandular stratified squamous epithelium near the lower esophageal sphincter (gastro-esophageal mucosa). In the glandular mucosa, the cardiac mucosa covers the proximal region, followed by the proper gastric mucosa covering a slightly smaller region, and the pyloric mucosa covers the glandular region near

the pyloric opening. The stomach of the horse is similar to the pig, except that the non-glandular stratified squamous epithelium is more extensive and covers the proximal one third. There is a smaller cardiac mucosal region and a region where the non-glandular and the glandular mucosa meet known as the margo plicatus. This region is where most gastric ulcers occur in horses. The horse has a similar size proper gastric and pyloric mucosa in the distal portion of the stomach[11].

Histology of the monogastric stomach

The stratified squamous epithelium in the forestomachs of the ruminant has an absorptive function. This tissue plays a major role in absorbing short chain fatty acids and NaCl. However, in the horse and pig non-glandular mucosa is thickened and cornified and has no absorptive or secretive function.

The glandular portion of the stomach is lined with simple columnar epithelium and goblet cells. Goblet cells produce a layer of mucous that provides mucosal protection. Gastric pits are visible on the mucosal surface and their walls are comprised of goblet cells and open into gastric glands (Fig I.2)[12]. These gastric glands contain different cell types depending on location within the stomach. The cardiac mucosa contains primarily mucus and bicarbonate secreting cells. Mucus is secreted from specialized mucus neck cells within the gastric pit and is a viscous, hydrophobic gel containing glycoproteins. Mucus adheres to the gastric

surface and provides protection to the underlying cells and lubrication to prevent mechanical damage from friction caused by course feedstuffs[13].

The proper gastric mucosa contains glands that are made up primarily of parietal or oxyntic cells and chief cells. Parietal or oxyntic cells secrete hydrochloric acid (HCl) [11,14]. Horses are continuous HCl secretors. Acid is thought to be the primary cause of gastric ulcers in horses; Murray in 1991 concluded that HCl and pepsin were the most important factors in causing gastric mucosal ulcerations[15].

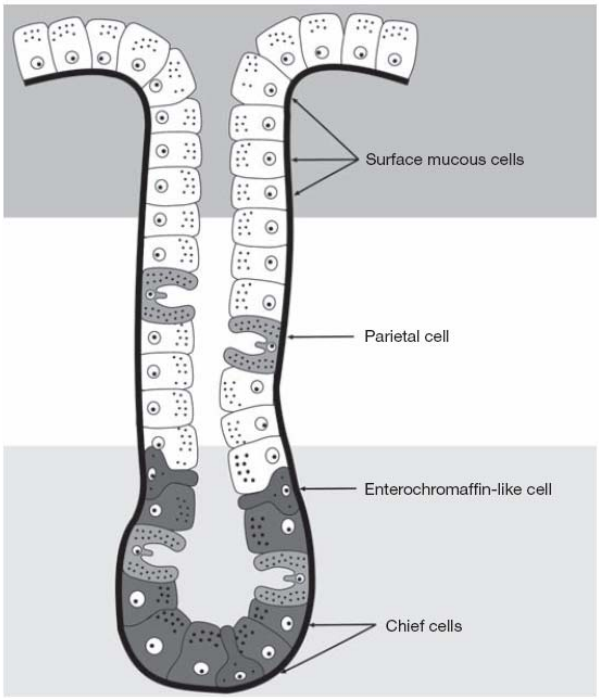

Figure I.2 Structure of gastric pit that opens into the gastric gland and the different cell types[12]

The HCl is produced by the parietal cell by secreting H^+ via H^+/K^+ ATPase or proton pump located on its apical membrane. The K^+ is exchanged for hydrogen and chloride as HCl by the proton pump and is secreted via ion-specific channels on the same apical membrane. The resulting HCl travels up the gastric gland into the gastric lumen, thereby increasing the acid content of the stomach. The gastric pits within the proper gastric mucosa also contain peptic or chief cells that secrete pepsinogen [11]. Peptic or chief cells when stimulated by histamine, gastrin, acetylcholine or secretin release HCl and stored pepsinogen. Acetylcholine and secretin stimulate the peptic cells to synthesize and secrete new pepsinogen. Pepsinogen is cleaved and activated to pepsin by gastric acid at the optimal pH of 2. After activation, pepsin quickly breaks down dietary proteins to peptides by cleaving the aromatic amino acid residues. Pepsin continue to digest proteins in an acidic environment, however it is inactivated when gastric juice pH increases to $\geq$ 4.0 or it is neutralized in the duodenum by bicarbonate secretion[13]. Interspersed within the proper gastric mucosa are enterochromaffin-like cells that release histamine and D cells that contain somatostatin.

These G and D cells in the pyloric mucosa help regulate gastrin secretion. Gastrin secretion is primarily stimulated by proteins present in the stomach after feeding. After the detection of proteins by the G cells, the gastrin-induced secretions of acid can occur in two pathways. Gastrin

stimulates the release of histamine by the enterochromaffin like cells, which then stimulates the parietal cells to secrete HCl, or to a lesser extent, there is direct stimulation of the parietal cell by gastrin. Somatostatin, produced by D cells, inhibits gastrin and thus gastric acid secretion.

Physiology of gastric secretions

Gastric secretions are controlled by both neural and hormonal mechanisms and consist of three phases. The cephalic phase prepares the stomach for the arrival of food by increasing gastric acid secretions. The sight, smell and taste of feed stimulate the parasympathetic nervous system via the vagus nerve to the submucosal plexus in the wall of the stomach. Postganglionic parasympathetic fibers that innervate the mucus neck cells, parietal cells, chief cells and the G cells are stimulated which results in an increase of secretions. This phase is short, lasting only minutes[16].

The second phase, or gastric phase, is much longer, lasting several hours and functions to enhance gastric acid secretions. This phase begins with the arrival of food into the stomach. Gastric secretion is stimulated and accounts for about two thirds of the total secretions. The gastric phase can be stimulated by the physical distension of the stomach, increase in stomach pH and the presence of undigested feed. Distension of the stomach activates stretch receptors which send signals to the

myenteric plexus and the medulla via the vagus nerve. This pathway results in the release of acetylcholine which stimulates gastric secretions. The increase in pH (>2) is attributed to the presence of proteins and other partially digested feed entering the stomach acting as a buffer; thereby directly stimulating the G cells to secrete gastrin. Gastrin primarily stimulates HCl production and to a lesser extent increases pepsinogen secretion[16].

The third phase or intestinal phase last several hours and functions to control the gastric emptying rate. This phase is under neural and hormonal control. The enterogastric reflex (neural control) inhibits gastrin production and gastric motility while stimulating contraction of the pyloric sphincter. This phase is activated by stretch receptors and chemoreceptors present in the pylorus. The arrival of medium- and long-chain fatty acids, amino acids (especially tryptophan) and carbohydrates into the small intestine stimulate the release of cholecystokinin (CCK) and gastric inhibitory peptide (GIP). The CCK inhibits gastric secretion of acid and pepsinogen and inhibits gastric emptying. The GIP inhibits gastric motility and secretion. These reflexes slow gut motility to allow sufficient time for digestion and absorption of nutrients and keep the small intestine from receiving gastric juice with an excessively low pH, which can be irritating[16].

Gastric Ulcers in Horses

Gastrointestinal ulcers in horses are defined by the Equine Gastric Ulcer Council as an alteration of the gastrointestinal mucosa that destroys cellular elements, resulting in damage that could extend to the level of the *lamina propria*. Less severe damage is referred to as erosion[17]. Equine gastric ulcer syndrome (EGUS) is the name given to the disease complex that is associated with ulceration of the esophageal, gastric or duodenal mucosa[17]. EGUS in horses is common and causes poor performance, which has a far-reaching economic impact within the horse industry. The horse industry contributes $102 billion yearly to the U.S. economy[18]. The exact economic impact of EGUS is not known; however the prevalence of gastric ulcers ranges from 40% to 93%, of which approximately 50% of affected horses showing clinical signs of colic and weight loss[19-21]. The high prevalence of gastric ulcers has been attributed to the anatomy of the horse stomach, diet, restricted feed intake and environmental stresses and is likely caused by exposure to hydrochloric and organic acids, such as VFAs (acetic, propionic, butyric, and valeric acids) and bile acids[10,22,23] .

Anatomy

The equine stomach anatomy predisposes it to gastric ulcer disease. The proximal one third of the equine stomach is lined with non-glandular stratified squamous epithelium. About 80% of ulcers are found in this region. This non-glandular mucosa is predisposed to acid injury due

to its lack of protective factors such as a bicarbonate-rich mucous layer[15]. The distal two thirds of the equine stomach is covered by glandular mucosa and is responsible for the secretion of mucus, HCl and pepsinogen[13]. This glandular region contains mucous-secreting cells and gastric glands that provide extensive protective mechanism, such as a bicarbonate-rich mucous layer. Approximately 20% of ulcers are found in this region, however the glandular region has an extensive capillary network which allows many of the ulcers to heal rapidly without intervention[17].

Diet

While acid has been implicated in the cause of gastric ulcers in horses, diet has been shown to be a risk factor. Size and composition of the grain meal is thought to have a profound effect on risk for developing EGUS[24]. It has been shown that high-energy rations containing high concentrations of soluble carbohydrates, like grains, or pelleted feeds produced a higher and more prolonged gastrin secretion than hay alone[25]. In addition grain diets are emptied rapidly from the stomach, when serum gastrin concentrations are highest[26].

Furthermore, concentrates or grain diets are high in soluble carbohydrates, which are fermented by resident bacteria, resulting in the production of VFAs. In the presence of low stomach pH (≤ 4), VFAs cause acid damage to the non-glandular squamous mucosa[27-29].

However, in a recent study, horses fed alfalfa hay and a pelleted concentrate diet had lower gastric ulcer scores than horses fed coastal bermuda grass hay[30]. Furthermore, a previous study showed that horses fed alfalfa hay and grain had a higher stomach pH and lower ulcer scores when compared to horses fed brome grass hay without grain[10]. In this study, the authors speculated that calcium and protein, both high in the alfalfa hay-grain diet, buffered stomach contents, resulting in a protective effect on the non-glandular mucosa. Thus, alfalfa hay when fed with or without concentrates may have a protective and anti-ulcer effect in horses.

Restricted Feed Intake

Several studies evaluated the effect of restricted feed intake on the development of gastric ulcers. Horses grazing at pasture have a decreased prevalence of gastric ulcers. During grazing, there is a continuous flow of saliva and ingesta that buffer stomach acid causing the stomach pH to be ≥ 4.0 for a large portion of the day. On the other hand, when feed is withheld from horses before a competition or as when stabled, gastric pH drops rapidly, and the non-glandular mucosa is exposed to an acid environment. However, a recent study showed that pastured pregnant and non-pregnant mares had a high prevalence of gastric ulcers[31]. The high prevalence of gastric ulcers in these pastured mares may be because horses consume less forage during evening hours than daytime hours, which may result in less saliva production and a low

pH environment in the proximal stomach; as was shown in a recent study showed that proximal stomach pH was lower in the early morning hours (1:00 am to 9:00 am) regardless of housing (paddock or stable) [32]. This may be why both pastured and stabled horses are susceptible to EGUS. Still, intermittent feeding has been shown to cause and increase the severity of non-glandular ulcers in horses, and is the concept behind a feed/fast model that has been used to consistently produce ulcers in research settings[33,34].

Environmental Stresses

Some of the environmental risk factors for EGUS include stall confinement, transport[35], and exercise[35,36]. Stall confinement in unfamiliar surroundings has been implicated as a risk factor for EGUS. Traditionally horses that are kept in pastures were thought to have a decreased prevalence of ulcers than those kept in stalls due to the continuous grazing and the constant flow of saliva. The reason for this may be multifactorial because horses that are stalled are often fed intermittently and housed alone. However, a recent study showed that neither proximal nor ventral stomach pH changed significantly in horses housed in stalls alone, housed in stalls with a companion, or housed in a grass paddock[32]. Here again, pH in the proximal stomach was lower during the early morning hours regardless of housing, and feed intake was lowest during these hours. Thus, other factors in stabled horses may increase their risk

of developing EGUS. For example, stabled horses are bolus fed (two large meals daily). These meals are traditionally high in grains and consumed rapidly, which leads to a decrease in saliva production and less buffering of stomach contents. Also, high grain diets may be fermented by resident stomach bacteria to VFA, which in an acid environment may lead to ulceration[10,27-29].

Horses involved in training and racing are at high risk to develop EGUS[37]. In fact, current prevalence figures show that 60% to 90% of performance race horses have EGUS[20,21,38,39]. A recent study showed that horses running on a high-speed treadmill have increased abdominal pressure and decreased stomach volume[40]. The authors' speculated that high intensity exercise allowed contraction of the stomach, resulting in reflux of the acid from the ventral glandular region of the stomach into the non-glandular region, leading to injury. Therefore, daily exercise may increase the exposure of the non-glandular mucosa to acid, explaining the increased prevalence of gastric ulcers in horses in training and racing. Furthermore, an increase in serum gastrin concentration has been shown to occur in exercising horses[41]. This increase in serum gastrin may increase glandular HCl secretion, which, in turn, may lead to NG mucosal acid damage.

Helicobacter spp

Although *Helicobacter* spp have been shown to be an important cause of gastric ulcers in other species, they have not been cultured to date in the horse. *Helicobacter pylori* and other *Helicobacter* species have not been proven to cause EGUS, although recently, *Helicobacter*-like DNA has been isolated from the glandular and non-glandular stomach mucosa in horses[42,43]. The significance of *Helicobacter* spp. in horses as a cause of EGUS is unknown at this time, however it is likely not a significant cause of NG gastric ulcers.

Ussing Chamber System

The Ussing chamber is a tool used to measure the net ion transport across tissue. Mucosa is placed between the two halves of the chambers and then both sides are filled with an equal amount of fluid, such as NRS. With the two similar solutions bathing the tissues no net transfer of passive ions take place, except those transported by active means. This active transport of sodium produces a voltage difference (PD) across the epithelium and is measured using electrodes[44]. The electrodes are connected to an automatic voltage clamp that allows for the short circuiting of the current or short circuit current (Isc), which is the current necessary to nullify the PD across the tissues. The Isc corresponds to the net transport of sodium from mucosal to serosal side[45]. If dissimilar solutions are used a junction potential is created, which can be measured

then corrected for by calculating the corrected short circuit current (clsc)[44]. The Ussing chamber is a proven tool used by many researchers to study the movement of ions across tissue in the frog[44], rabbit[45,46], horse[9,27,28,47] and pig[7,48].

CHAPTER II. Objectives

Hypothesis: We hypothesize that the VFA mixes will alter the bioelectric properties of the equine NG mucosa, specifically by a decrease in PD, Isc and G, with an increase in R.

The purpose of this study was to determine the effects of a sub-threshold mixture of VFAs, found in gastric juice of horses after feeding, and three VFA mixtures containing above-threshold concentrations of a selected VFA, when exposed to equine NG mucosa causes changes in the bioelectric properties resulting in histopathologic changes of cellular swelling, lysis, and necrosis. The specific objectives include:

1. To measure the short circuit current (Isc) and potential difference (PD) across the tissue and calculate the conductance (G) and resistance (R) of NG mucosa from recently euthanatized horses exposed to a NRS, a sub-threshold VFA mixture, typically found 2 hours after feeding, and three mixtures containing VFAs above threshold, at different pHs (1.5, 4.0, 7.0) in an *in vitro* Ussing chamber system.
2. To examine the NG tissues, after exposure to the NRS and the VFA mixtures in NRS in objective 1, under light microscopy for histopathologic changes such as cell swelling and necrosis.

CHAPTER III. Materials and Methods

Animals and Treatments

The objectives were met in the Large Animal Clinical Sciences research laboratory at the University of Tennessee College of Veterinary Medicine (UTCVM) which has 15 Ussing chambers and the technical experience to perform the experiments. Eleven horses of either sex were donated to UTCVM for various reasons, which included lameness, various injuries, old age, or behavioral issues. Horses were humanely euthanatized by an injection of a lethal overdose of barbiturate (Beuthanasia®, Schering- Plough, Kenilworth, NJ). Stomachs were removed from the horses within 30 minutes of euthanasia and dissected along the greater curvature to expose the NG squamous mucosa. The NG squamous mucosa was then rinsed using NRS to remove adherent food material, in preparation for mounting in Ussing chambers. The stomach was then examined for the presence of bots and for gastric ulceration and given a score based on a previously published gastric ulcer scoring system [49]. The NG mucosal tissue from one horse was not mounted in Ussing chambers because it was covered with extensive ulceration.

A 3.5cm² diameter disc of NG squamous mucosa was mounted in Ussing chambers and exposed to different VFAs. Upon completion of the

VFA exposure, the tissue was removed from the chamber and placed in formalin for histopathologic examination.

Ussing Chamber Techniques

Ussing chamber techniques are based on previous studies performed by Argenzio and Eismann [48] and adopted to horse tissue [27-29,50]. The NG squamous mucosa was pinned mucosal side down in a paraffin dissecting tray bathed in an oxygenated Ringers solution at room temperature (20 to 22º C). The NG squamous mucosa was then sharply dissected from the underlying muscular layer and a 3.5cm² diameter disc (area of 7.07cm²) of mucosa was mounted in the Ussing chamber. The mucosal side was then perfused with a solution that contained either a sub-threshold VFA mix, high acetic acid VFA mix, high butyric acid VFA mix, high propionic acid VFA mix, or NRS (Table3-2) as the control at a pH of 1.5, 4.0 or 7.0. The sub-serosal surface of the tissues were bathed in the NRS at pH 7.0 and the tissues were oxygenated by circulating the solutions in a water-jacket reservoirs with a gas lift bubbled with a 5% CO_2-95%0_2. Spontaneous PD was measured by using agar bridges that were made from the NRS, and have an identical composition to the solution bathing the serosal tissue. The PD was nullified by the use of an automatic voltage clamp through Ag-AgCl electrodes. The Isc was determined as the current necessary to nullify the PD. Tissue R and G was calculated from the open-circuit PD. If the open-circuit PD was <1mv,

the tissue was clamped at 100uA of current and the resulting PD was recorded. Electrodes filled with and bathed in saturated KCl were used to record the PD and Isc.

Fifteen chambers were used in blocks of 3; with each block containing the pH 1.5, 4.0, and 7.0. For the study, control tissues were bathed in NRS, (Table III-1). For each experiment tissues and treatments; NRS, VFA mixtures (Table III-1) were randomly assigned to the blocks of 3 chambers. The NRS was added to the serosal side of the tissue at pH 7.0 and the solution containing the VFA mix at three pHs were added on the mucosal side. Although the two different baths were used to bathe the mucosal and serosal sides, liquid junction potentials were not observed during electrical measurements, so there was no adjustment in the PD.

Table III-1 Treatment solutions and the composition

Solution	**Concentration in mM**
Normal Ringers Solution	142 NA, 124 Cl, 25 HCO_3, 10 glucose, 5 K, 1.64 HPO_4, 1.25 Ca, 1.1 Mg 0.3 H_2PO_4
VFA Mix	21 acetic, 1.0 propionic, 1.5 butyric, 1.0 valeric, 5 D/L lactic acids
High Acetic Acid VFA	40 acetic, 1.0 propionic, 1.5 butyric, 1.0 valeric, 5 D/L lactic acids
High Butyric Acid VFA Mix	21 acetic, 1.0 propionic, 40 butyric, 1.0 valeric, 5 D/L lactic acids
High Propionic Acid VFA Mix	21 acetic, 40 propionic, 1.5 butyric, 1.0 valeric, 5 D/L lactic acids

The pH (1.5, 4.0, and 7.0) of the solutions that bathed the mucosal side of the tissue was adjusted by titration of 1M and 5M HCl while measuring with a portable pH electrode (Accumet portable pH meter, Fisher Scientific, Pittsburg Pa.). Fifteen chambers were used in blocks of 3; with each block containing the pH 1.5, 4.0, and 7.0. For each experiment the tissues and treatment; NRS, VFA mixture 1, 2, 3, or 4 (Table III-1) were randomly assigned to the blocks of 3 chambers. The PD and Isc were measured every 15 minutes during the 330-minute experimental period. The pH of the solutions were measured every hour and adjusted as needed during the experimental period.

The R and G were calculated based on Ohm's law; V (voltage) = I (current(amps)) x R (resistance(ohms)). The equations used were:

Conductance (G) = Isc/area of the chamber (7.07 cm^2)/ PD

Resistance (R) = (1/G) x 1000

Preparation for Histopathologic Examination

After exposure in Ussing chambers, mucosa was placed in 10% formalin and prepared for histopathlologic examination. Tissues were trimmed and embedded in paraffin, sectioned at 5 μm, placed on glass slides and stained with hematoxylin and eosin. Slides were then examined under light microscopy to determine amount of cellular swelling or necrosis present.

Statistical Analysis

Data were analyzed using a statistical computer program, SAS (SAS Institute, Cary, NC), using a randomized block design with repeated measures, ANOVA. Each horse was considered a block, VFA solutions and pH with all interactions were in the main plot, and time and the interactions of time with the main plot factors were in the subplot. Time was considered a repeated measure factor. Least square means (LSM) and standard error of the means were calculated and mean separation using a protected Fisher's LSD test was performed. Conductance and R were transformed using a log transformation to correct for normality. Values of $P<0.05$ were considered significant.

CHAPTER IV. Results

Animals and gastric tissue

Horses that were euthanized were donated to the University of Tennessee for various reasons. Horses (n=11) ranged from 1 to 26 years of age (mean, 12.4 years) and 3 of 11 (27%) of horses had gastric ulcers in the non-glandular mucosa. Only tissues from 10 horses could be evaluated in Ussing chambers, as the stomach of one horse had severe ulcers over most of the NG mucosa. Information collected on each horse was reported in the following tables; age (Table A.1), breed (Table A.1), sex (Table A.2), reason for euthanasia (Table A.3), *Gastroghilus intestinalis* larvae or bots present in on the mucosa (Table A.4), and the gastric ulcer score (Table A.5). The NG gastric ulcer score was determined for 3 horses that had gastric ulcers. They were scored from 1 to 3, with 1 being small focal or multifocal ulcers and 3 being extensive coalescing ulcers. The variables measured did not differ significantly between tissues from horses with ulcers and those without ulcers.

VFA Mixes and control exposure to mucosa

There was a significant interaction between time, pH and treatment for potential difference (PD) (P=0.001) at the lower pHs, but mean PD values at pH 7.0 in tissues exposed to NRS did not differ significantly from any of the VFA-treated tissues (Figure A.1).

Mean PD in tissues exposed to the high acetic acid VFA mix at pH 4.0 did not differ significantly from tissues exposed to NRS. Mean PD was significantly decreased in tissues exposed to the VFA mix when compared to tissues exposed to NRS from 150 minutes until 255 minutes. However, this difference may not have been physiologically significant because mean PD in tissues exposed to the VFA mix at pH 4.0 started out lower than tissues exposed to NRS and remained low throughout the experimental period. Mean PD in tissues exposed to the high propionic acid VFA mix was significantly lower from 105 until 210 minutes compared to tissue exposed to NRS solution at the same time points, whereas mean PD in the high butyric acid VFA mix was significantly lower from 75 minutes until the end of the experimental period when compared to tissue exposed to NRS at the same time points (Figure A.2).

Mean PD in tissues exposed to the high acetic acid VFA mix at pH 1.5 and the VFA mix did not differ significantly from mean PD in tissue exposed to NRS solution alone at the same pH. However, mean PD in tissues exposed to the high propionic acid VFA mix was significantly decreased when compared to tissue exposed to NRS at 150 minutes, whereas mean PD in tissues exposed to the high butyric acid VFA mix was significantly decreased compared to mean PD in tissues exposed to NRS at 60 minutes and remained significantly lower throughout the experimental period (Figure A.3). Mean PD in tissues exposed to NRS

and the VFA mixes significantly decreased after 30 minutes as the pH was decreased to 1.5.

Mean Isc in tissues exposed to the high propionic acid VFA mix was significantly lower when compared to tissues exposed to NRS at pH of 7.0 (Figure A.4). However, mean Isc in the high propionic acid VFA mix started significantly lower than the control tissue and continued to stay lower until 75 minutes into the experiment. If all of the tissues had started at the same point there would not be any significance at pH 7.0 of Isc. Thus, this was not considered a significant physiologic difference.

Mean Isc in tissues exposed to the high acetic acid VFA mix, high propionic acid VFA mix, and the VFA mix at pH 4.0 did not differ significantly from mean Isc in tissues exposed to the NRS at the same pH. However, mean Isc in the tissues exposed to the high butyric acid VFA mix significantly decreased after the pH was decreased to 4.0 and remained significantly lower from 75 until 285 minutes, compared to Isc in tissues exposed to NRS at the same pH. At 285 minutes tissues exposed to the high butyric acid VFA mix leveled off while the control tissue continued to gradually decrease (Figure A.5).

Mean Isc in tissues exposed to NRS was significantly different from all VFA treatments at some point over the 330 minute experimental period. Tissues exposed to NRS at pH 1.5 followed expected trends from 0 to 180 minutes, however at 210 minutes mean Isc decreased more than

expected for the experimental period. This dramatic decrease in mean Isc in tissues exposed to the NRS may have been caused by two horses that produced spurious data that were outliers when compared to the other eight horses in the study. If these data from the two horses were removed the graph of the man Isc in tissues exposed to NRS would be similar to data for tissues exposed to the VFA mix at the same pH.

Mean Isc for tissues exposed to the high acetic acid VFA mix at pH 1.5 was significantly lower that the tissue exposed to NRS from 120 until 165 minutes at the same pH. Tissues exposed to the VFA mix was significantly different from the tissues exposed to the NRS at pH 1.5 from 225 to 330 minutes. Mean Isc in tissues exposed to the high propionic acid VFA mix was significantly lower than the tissues exposed to NRS from 75 until 180 minutes, while mean Isc in tissues exposed to the high butyric acid VFA mix was significantly lower than mean Isc in tissues exposed to NRS at pH 1.5 from 60 until 300 minutes (Figure A.6).

There was a not a significant interaction between time, pH and treatment for resistance (R) (P=0.825). A log transformation was conducted on the original data to help with normality and the log transformed values are represented in the graphs at pH 7.0 (Figure A.7), pH 4.0 (Figure A.8), and pH 1.5 (Figure A.9). While no significant differences were noted in mean R, data for all treatments at pH 7.0 and

pH 4.0 increased slightly from 0 to 330 minutes. Whereas, R remained lower at pH of 1.5 over the 330 minutes experimental period.

There was a not a significant interaction between time, pH, and treatment for conductance (G) (P=0.623). A log transformation was conducted on the original data to help with normality and the log transformed values are represented in the graphs at pH 7.0 (Figure A.10), pH 4.0 (Figure A.11), and pH 1.5 (Figure A.12). While no significant differences were noted in mean G at any pH, tissues exposed to pH 1.5 showed a lower G than tissues exposed at the pH 4.0 and 7.0.

The interaction between pH and time was significant (P= <0.0001) for PD, Isc, R and G. Mean PD in tissues exposed to NRS, at 1.5 was significantly lower than tissue exposed to NRS at pH 4.0 and pH 7.0 from 45 minutes until the end of the experimental period. Also, mean PD in tissues exposed to NRS at pH 4.0 was significantly lower at 45 minutes and again from 90 to 330 minutes, when compared to tissues exposed to NRS at pH 7.0 (Figure A.13). Mean Isc in tissues exposed to NRS at pH 1.5 was significantly decreased compared to mean Isc in tissues exposed to NRS at pH 4.0 and 7.0 from 45 minutes until the end of the experimental period. Also, mean Isc in tissues exposed to NRS at pH 4.0 was significantly lower than mean Isc in tissues exposed to NRS at pH 7.0 from 30 minutes, at 45 minutes and then again from 75 to 330 minutes (Figure A.14). Mean R in tissues exposed to NRS at pH 1.5 was

significantly lower than tissue exposed to NRS at pH 7.0 from 105 until 330 minutes. Also, mean R in tissues exposed to pH 1.5 was significantly lower than mean R in tissues exposed to pH 4.0 and 7.0 from 60 minutes until the end of the study (Figure A.15). Mean G in tissues exposed to NRS at pH 1.5 was significantly lower than mean G in tissues exposed at pH 7.0 from 105 to 330 minutes of the experimental period. Mean G in tissue exposed to pH 1.5 was significantly lower in tissues exposed to pH 1.5 from 60 minutes to 330 minutes when compared to pH 4.0, while tissues exposed to NRS at pH 4.0 was significantly lower than tissues exposed at pH 7.0 from 135 to 255 minutes (Figure A.16).

There was a significant interaction between the time, pH and treatment for PD and Isc (P=0.001). Mean PD in tissues exposed to NRS at pH 1.5 was significantly lower than tissues exposed to NRS at pH 4.0 and 7.0 from 45 minutes until the end of the experimental period. However, there was no significant difference in mean PD in tissues exposed to NRS at pH 4.0 and 7.0 (Figure A.17). Mean PD in tissues exposed to the high acetic acid VFA mix at pH 1.5 was similar to results above in tissue exposed to NRS alone at the same pH. Mean PD in tissues exposed to the high acetic acid VFA mix at pH 1.5 was lower than mean PD in tissues exposed at pH 4.0 and 7.0, from 45 until 330 minutes and from 60 to 330 minutes. While, mean PD in tissues exposed to the high acetic acid VFA mix at pH 4.0 and 7.0 were not significantly different

from each other (Figure A.18). Mean PD in tissues exposed to the high butyric acid VFA mix at pH 4.0 was significantly decreased when compared to mean PD in tissues exposed at pH 7.0 from 75 to 330 minutes. Mean PD in tissues exposed to the high butyric acid VFA mix at pH 1.5 was significantly lower than mean PD in those tissues exposed at pH 4.0 and 7.0 from 45 minutes to the end of the experimental period (Figure A.19). Mean PD in tissues exposed to the high propionic acid VFA mix at pH 1.5 was significantly decreased compared to mean PD in tissues exposed to the high propionic acid VFA mix at pH 7.0 from 75 minutes until the end of the experimental period. Also, mean PD in tissues exposed to the high propionic acid VFA mix at pH 1.5 was significantly lower than mean PD in tissues exposed at pH 4.0 from 60 minutes until the end of the experimental period (Figure A.20). Mean PD in tissues exposed to the VFA mix at pH 1.5 was significantly decreased from 45 minutes to the end of the experimental period when compared to mean PD in tissues exposed to the VFA mix at pH 7.0. Whereas, mean PD in tissues exposed to the VFA mix at pH 1.5 were significantly lower than mean PD in tissues exposed to the VFA mix at pH 4.0 from 195 minutes to 330 minutes. Furthermore, mean PD in tissues exposed to the VFA mix at pH 4.0 were significantly decreased at 45 minutes and again from 105 to 210 minutes, when compared to mean PD in tissues exposed to the VFA mix at pH 7.0. These changes were not physiologically

different, as mean PD in tissues exposed to the VFA mix at pH 4.0 was lower at the start of the experiment and remained lower throughout the experiment (Figure A.21).

There was a significant interaction between the time, pH and treatment for Isc (P=0.001). Mean Isc in tissues exposed to NRS at pH 1.5 was significantly decreased at 45, from 75 and 105 minutes and then again from 180 to 330 minutes, when compared to tissues exposed to NRS at pH 7.0. The unexpected sharp decrease at 105 minutes for pH 1.5 could be explained by outliers within the data from 2 horses. There was a significant difference in mean Isc in tissues exposed to NRS between pH1.5 and 4.0 from 225 minutes until 330 minutes; however there are no significant differences in tissues exposed to NRS at pH 4.0 when compared to tissues exposed to NRS at pH 7.0 (Figure A.22). Mean, Isc in tissues exposed to the high acetic acid VFA mix at pH 1.5 was significantly decreased from 75 minutes until the end of the experimental period, when compared to tissues exposed to the high acetic acid VFA mix at pH 7.0. Mean Isc in tissues exposed to the high acetic acid VFA mix at pH 1.5 was significantly decreased from 90 until 210 minutes and again from 225 to 330 minutes, when compared mean Isc in tissues exposed to pH 4.0. Mean Isc in tissues exposed to the high acetic acid VFA mix at pH 4.0 did not differ from those tissues exposed to the same solution at pH 7.0 (Figure A.23). Mean Isc in tissues exposed to the

high butyric acid VFA mix at pH 1.5 was significantly decreased from 45 minutes to the end of the experimental period, when compared to the mean Isc in tissues exposed at pH 4.0 and 7.0. Mean Isc in tissues exposed to the high butyric acid VFA mix at pH 4.0 was significantly decreased from 70 to 300 minutes when compared to the mean Isc in tissues exposed to the same solution at pH 7.0 (Figure A.24). Mean Isc in tissues exposed to the high propionic acid VFA mix at pH 1.5 was significantly decreased when compared to mean Isc in tissues exposed to the same solution at pH 4.0 and 7.0 from 75 minutes until 330 minutes. However, mean Isc in tissues exposed to the high propionic acid VFA mix did not differ significantly in tissues exposed at pH 4.0 and pH 7.0 (Figure A.25). Mean Isc in tissues exposed to the VFA mix did not significantly differ at any pH (Figure A.26).

Mean R (P=0.825) and G (P=0.623) in tissues exposed to the NRS and the VFA mixes did not differ significantly at any pH. A log transformation was used to correct for normality and the log transformed values are represented in the graphs at each treatment with all pHs (1.5, 4.0 and 7.0): Figure A.27, Figure A.28, Figure A.29, Figure A.30, Figure A.31, Figure A.32, Figure A.33, Figure A.34, Figure A.35, and Figure A.36.

CHAPTER V. Discussion

Animals and gastric tissue

Prevalence of gastric ulcers in horses in this study was 27%, with the mean ulcer score being 2.0 on a scale of 0 to 3. These findings were lower than previous reports[27-29] where prevalence of gastric ulcers was 46%, 53% and 56% respectively. The high prevalence of gastric ulcers in these previous reports was explained by older horses that were donated because underlying medical conditions and lameness. The low prevalence of gastric ulcers in this study may be explained by the small population of horses (n=11) compared to previous studies where there was a larger number of horses. Also, horses in this study suffered from different conditions than previous studies and may not have had the types of conditions that lead to gastric ulcers.

VFA Mixes and control exposure to mucosa

Results of this study indicate there was no significant effect of the VFA mix or the high acetic acid VFA mix on tissue bioelectric properties in equine NG mucosa at pH 1.5, 4.0 or 7.0, other than what was seen by exposure to HCl alone. Thus, we partially reject the hypothesis that the VFA mix and the high acetic acid VFA mix caused alterations in the bioelectric properties of NG mucosal tissues. The VFA mix likely did not have a significant effect on NG tissue bioelectric properties because the

VFA concentrations in the mix were at or below threshold concentrations as previously described[29]. With VFA concentrations at or below threshold concentrations, tissues exposure of 330 minutes likely does not cause functional changes in NA^+ transport, R and G across NG tissues except what was seen with a change in pH alone.

Also, there were no changes in the bioelectric properties of tissues exposed to the high acetic acid VFA mix. This was similar to previous reports in horses, where tissues exposed to a 40 mM concentration of acetic acid did not show a significant reduction in bioelectric peoperties[29]. However, because acetic acid is found in the highest concentration in gastric juice 2 hours after feeding, a longer exposure time may be required for acetic acid at a concentration of 40 mM to cause significant changes in NG tissues.

Tissues exposed to the high propionic acid and the high butyric acid VFA mixes showed a significant decrease in tissue Isc and PD. The greatest decrease in bioelectric properties occurred with the high butyric acid VFA mix at pH 1.5, compared to tissue exposed to the same solution at pH 4.0 and 7.0. In this study, NG tissue exposed to VFA mixtures containing high concentrations of butyric and propionic acid at pH ≤ 4.0 induced functional mucosal damage manifested as a decrease in sodium transport (Isc), a decrease in PD, with an increase in tissue R and decrease in tissue G. There was no histological evidence of cellular

damage for the VFA mix; however there was evidence of cellular swelling in tissues exposed to the high propionic and high butyric acid VFA mixtures. Thus we accept the hypothesis that VFA mixes with high concentrations of propionic and butyric acids caused changes in bioelectric properties in NG mucosa.

Changes in the bioelectric measurements of the NG mucosa in horses are based on the results of previous studies[45,48,51,52] on tissues from other organs and species. Values for Isc, PD and R are somewhat similar to what was found in other studies for equine NG mucosa exposed to HCl and VFAs[27-29]. The Isc is a direct indicator of active sodium ion transport and therefore a useable measurement of epithelial function and tissue viability. Experiments with frog skin,[51] rumen epithelium,[53] and rabbit esophagus[45] showed that Isc is equal to the net Na+ transport across tissues. In rumen epithelium and rabbit esophageal epithelium, it was found that the viable layers beneath the stratum corneum are involved in Na^+ transport. This transport is due the high density of intracellular Na^+ pumps (Na^+ K^+ ATPase) in these tissues.[45,53] Also results of studies by Goldstein et al[46,54] and Snow et al[54] indicate that rabbit esophageal cells of the stratum spinosum have pH-sensitive basolateral potassium channels. The acidification of rabbit esophageal tissues resulted in inhibition of the potassium channels and abolished Isc, which in turn prevented the regulatory volume decrease usually seen after

hypotonic medium-induced cellular swelling. In another study it was shown that basolateral potassium channels are present in the equine NG mucosal cells and that these channels are important for active Na^+ absorption across the apical surface of the epithelium, control of intracellular pH and maintenance of cell volume[47]. Inhibition of these channels by acidification (pH 1.7) abolishes Isc and PD and decreases R, which in turn makes cells more susceptible to damage by HCl. Thus, cellular Na^+ K^+ ATPase and basolateral potassium channels in the equine NG mucosa may play a major role in maintaining cellular fluid volume, pH, and barrier function.

In the study presented here, Isc in tissues perfused with NRS at a pH of 1.5 and 4.0 significantly decrease after 45 minutes of exposure. The PD at pH 4.0 significantly decreased at 45 minutes then rebounded to pretreatment levels, while PD in tissues exposed to NRS at pH 1.5 significantly dropped after 45 minutes. The mean R was significantly lower at pH 1.5 than pH 7.0 from 105 minutes until the end of the study while the G was significantly decreased at 105 minutes. These data suggest that hydrogen ions cause an increase in outer barrier permeability, resulting in a decrease in PD and Isc and later a decrease in R. Hydrogen ions diffuse into the deeper sodium-transporting cell layers (ie, stratum transitionale and stratum spinosum), which are then acidified,

causing in a subsequent decrease in sodium transport (decrease in Isc) and eventually cell swelling.

However, Isc in tissues exposed to NRS at pH 1.5 showed a sharp decrease at 180 minutes and continued until the end of the experimental period. This was not observed in previous studies and could be explained by two horses in the study with data that was found outside the normal data. This aberrant data may have been caused by mechanical malfunction of the Ussing chamber system, and if removed, data would follow the trend of the tissue exposed to the VFA mix at the same pH.

In contrast to the lack of significant decrease in tissue PD and Isc when exposed to the high acetic acid VFA mix, tissues exposed to the high butyric acid and high propionic acid VFA mixes at pH 1.5 and 4.0 showed an immediate and significant decrease in sodium transport. This significant decrease in sodium transport caused by a 40 mM concentration of propionic and butyric acids is consistent with previous reports in the literature, where NG tissues exposed to these individual VFAs at the same concentration showed a dramatic chain-length decrease in sodium transport[27]. However, this is the first study that showed this decrease in Isc when tissue was exposed to a mixture of VFAs, similar to that found in the horse stomach after consuming a high grain diet.

The data for R and G were log transformed to correct for lack of normality. While not significant there was an increase in R in

tissues exposed to the high propionic acid and thigh butyric acid VFA mixes. This finding was similar to previously reported findings in tissue exposed to a 40 mM concentration of these individual VFAs, with the data in the un-transformed state[27,28]. For R, at pH 1.5 the tissues remained decreased when compared to tissues exposed to the high propionic and butyric acid mixes at pH 4.0 and 7.0. There was a slight increase in tissue R which is likely explained by tissues acclimating to the Ussing chamber system. There was not a significant decrease in G; however these findings were similar to previous reported findings for NG mucosa with the data in the un-transformed state[27,28]. The G remained increased in tissues exposed to the high propionic and butyric acid VFA mixes at pH 1.5, while G decreased in the same tissues exposed to the same solutions at pH 4.0 and 7.0 for the experimental period.

Histologic changes (primarily cellular swelling) were most severe in the tissues exposed to the high butyric acid VFA mix at a pH of 1.5, when compared to the same tissues exposed to pH 4.0 or 7.0. In addition, there was some evidence of cellular swelling in tissue exposed to the high propionic acid VFA mixture at pH of 1.5.

Analysis of results of the study suggest that a sub-threshold mixture of VFAs (<40 mM VFAs), do not cause changes in bioelectric properties of equine NG mucosa, when exposed over 330 minutes, other than the changes seen with HCl alone. However, if the concentrations of VFAs

exceed 20 mM or the threshold level, especially for propionic and butyric acids, it will lead to changes in the NG tissue barrier function, which could result in ulcer formation. The VFA-induced injury in the presence of HCl is likely due to damage to cellular sodium transport in the living layers just beneath the stratum corneum, which leads to eventual cell swelling, sloughing and ulceration. These data presented in this study suggest that horses fed fermentable carbohydrates that generate VFAs, when exposed to the NG mucosa at sub-threshold concentrations (<40 mM) do not caused alterations in NG mucosal bioelectric properties and may not lead to gastric ulcers. Whereas, high concentrate diets that produce gastric juice VFA concentrations of >20 mM, especially propionic and butyric acids will likely lead to acid injury and ulceration.

Literature Cited

1. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 1990;70:567-590.

2. Bugaut M. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comp Biochem Physiol B* 1987;86:439-472.

3. France J, Siddons RC. Volatile Fatty Acid Production In: J. M. Forbes JF, ed. *Quantitative Aspects of Ruminant Digestion and Metabolism*. Wallingford, UK: C. A. B. International, 1993;107-121.

4. Herdt T. Gastriontestinal Physiology and Metabolism In: Cunningham JG, ed. *Textbook of Veterinary Physiology*. Philadelphia: W. B. Saunders Company, 2002;222-322.

5. Stewart WE, Stewart DG, Schultz LH. Rates of volatile fatty acid production in the bovine rumen. *Journal of Animal Science* 1958;73:723-736.

6. Elsden SR, Hitchcock MWS, Marshall RA, et al. Volatile acid in the digesta of ruminants and other animals. *Journal of Experimental Biology* 1946;22:191-202.

7. Argenzio RA, Southworth M. Sites of organic acid production and absorption in gastrointestinal tract of the pig. *Am J Physiol* 1975;228:454-460.

8. Banta CA, Clemens ET, Krinsky MM, et al. Sites of organic acid production and patterns of digesta movement in the gastrointestinal tract of dogs. *J Nutr* 1979;109:1592-1600.

9. Argenzio RA, Southworth M, Stevens CE. Sites of organic acid production and absorption in the equine gastrointestinal tract. *Am J Physiol* 1974;226:1043-1050.

10. Nadeau JA, Andrews FM, Mathew AG, et al. Evaluation of diet as a cause of gastric ulcers in horses. *Am J Vet Res* 2000;61:784-790.

11. Reece WO. *Dukes' Physiology of Domestic Animals*. 12th ed. Ithaca, NY: Cornell University Press, 2004.

12. Bell RJ, Mogg TD, Kingston JK. Equine gastric ulcer syndrome in adult horses: a review. *N Z Vet J* 2007;55:1-12.

13. Sernka TJ, Jacobson ED. *Gastrointestional Physiology - the essentials*. 2nd ed. Baltmore, MD: Williams & Wilkins, 1983.

14. Sachs G, Hersey SJ. *The Gastric Parietal Cell*. Oxford, England: Oxford Clinical Communications, 1990.

15. Murray MJ. The pathogenesis and prevalence of gastric ulceration in foals and horses. *Veterinary Medicine* 1991:851-819.

16. Akers RM, Denbow DM. *Anatomy and Physiology of Domestic Animals*. 1 ed. Ames, Iowa: Blackwell Publishing 2008.

17. Anon. Recommendations for the diagnosis and treatment of equine gastric ulcer syndrome (EGUS). *Equine Veterinary Education* 1999;1:122-134.

18. Anon. National Economic Impact Study of the Horse Industry. Washington DC: Deloitte Consulting LLP, 2005.

19. Bertone JJ. Prevalence of Gastric Ulcers in Elite, Heavy Use Western Performance Horses. American Association of Equine Practioners 2000;256-259.

20. Murray MJ, Schusser GF, Pipers FS, et al. Factors associated with gastric lesions in thoroughbred racehorses. *Equine Vet J* 1996;28:368-374.

21. Hammond CJ, Mason DK, Watkins KL. Gastric ulceration in mature thoroughbred horses. *Equine Vet J* 1986;18:284-287.

22. Geor RJ, Harris PA. How to minimize gastrointestinal disease associated with carbohydrate nutrition in horses. American Association of Equine Practitioners 2007;178-185.

23. Andrews FM, Buchanan BR, Elliott SB, et al. Gastric ulcers in horses. *Journal of Animal Science* 2005;83(Suppl):E18-E21.

24. Metayer N, Lhote M, Bahr A, et al. Meal size and starch content affect gastric emptying in horses. *Equine Vet J* 2004;36:436-440.

25. Smyth GB, Young DW, Hammond LS. Effects of diet and feeding on postprandial serum gastrin and insulin concentration in adult horses. *Equine Vet J Suppl* 1989:56-59.

26. Sandin A, Girma K, Sjoholm B, et al. Effects of differently composed feeds and physical stress on plasma gastrin concentration in horses. *Acta Vet Scand* 1998;39:265-272.

27. Nadeau JA, Andrews FM, Patton CS, et al. Effects of hydrochloric, valeric, and other volatile fatty acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *Am J Vet Res* 2003;64:413-417.

28. Nadeau JA, Andrews FM, Patton CS, et al. Effects of hydrochloric, acetic, butyric, and propionic acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *Am J Vet Res* 2003;64:404-412.

29. Andrews FM, Buchanan BR, Smith SH, et al. In vitro effects of hydrochloric acid and various concentrations of acetic, propionic, butyric, or valeric acids on bioelectric properties of equine gastric squamous mucosa. *Am J Vet Res* 2006;67:1873-1882.

30. Lybbert T, Gibbs P, Cohen N, et al. Feeding Alfalfa Hay to Exercising Horses Reduces the Severity of Gastric Squamous Mucosal Ulceration. American Association of Equine Practitioners 2007;525-226.

31. le Jeune SS, Nieto JE, Dechant JE, et al. Prevalence of gastric ulcers in Thoroughbred broodmares in pasture: A preliminary report. *Vet J* 2008.

32. Husted L, Sanchez LC, Olsen SN, et al. Effect of paddock vs. stall housing on 24 hour gastric pH within the proximal and ventral equine stomach. *Equine Vet J* 2008.

33. Murray MJ. Equine model of inducing ulceration in alimentary squamous epithelial mucosa. *Dig Dis Sci* 1994;39:2530-2535.

34. Murray MJ, Schusser GF. Measurement of 24-h gastric pH using an indwelling pH electrode in horses unfed, fed and treated with ranitidine. *Equine Vet J* 1993;25:417-421.

35. McClure SR, Carithers DS, Gross SJ, et al. Gastric ulcer development in horses in a simulated show or training environment. *J Am Vet Med Assoc* 2005;227:775-777.

36. Buchanan BR, Andrews FM. Treatment and prevention of equine gastric ulcer syndrome. *Vet Clin North Am Equine Pract* 2003;19:575-597.

37. Vatistas NJ, Sifferman RL, Holste J, et al. Induction and maintenance of gastric ulceration in horses in simulated race training. *Equine Vet J Suppl* 1999:40-44.

38. Vatistas NJ, Snyder JR, Carlson G, et al. Cross-sectional study of gastric ulcers of the squamous mucosa in thoroughbred racehorses. *Equine Vet J Suppl* 1999:34-39.

39. Begg LM, O'Sullivan CB. The prevalence and distribution of gastric ulceration in 345 racehorses. *Aust Vet J* 2003;81:199-201.

40. Lorenzo-Figueras M, Merritt AM. Effects of exercise on gastric volume and pH in the proximal portion of the stomach of horses. *Am J Vet Res* 2002;63:1481-1487.

41. Furr M, Taylor L, Kronfeld D. The effects of exercise training on serum gastrin responses in the horse. *Cornell Vet* 1994;84:41-45.

42. Contreras M, Morales A, Garcia-Amado MA, et al. Detection of Helicobacter-like DNA in the gastric mucosa of Thoroughbred horses. *Lett Appl Microbiol* 2007;45:553-557.

43. Scott D, Marcus E, Shirazi-Beechey S. Evidence of Helicobacter infection in the horse. Proceedings of the American Society of Microbiologists 2001;287.

44. Ussing HH, Zerahn K. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Reprinted from Acta. Physiol. Scand. 23: 110-127, 1951. *J Am Soc Nephrol* 1999;10:2056-2065.

45. Powell DW, Morris SM, Boyd DD. Water and electrolyte transport by rabbit esophagus. *Am J Physiol* 1975;229:438-443.

46. Goldstein JL, Fogelson BG, Snow JC, et al. Rabbit esophageal cells possess K+ channels: effect of hyposmotic stress on channel activity. *Gastroenterology* 1993;104:417-426.

47. Widenhouse TV, Lester GD, Merritt AM. Effect of hydrochloric acid, pepsin, or taurocholate on bioelectric properties of gastric squamous mucosa in horses. *Am J Vet Res* 2002;63:744-749.

48. Argenzio RA, Eisemann J. Mechanisms of acid injury in porcine gastroesophageal mucosa. *Am J Vet Res* 1996;57:564-573.

49. MacAllister CG, Andrews FM, Deegan E, et al. A scoring system for gastric ulcers in the horse. *Equine Vet J* 1997;29:430-433.

50. Andrews FM, Buchanan BR, Elliott SB, et al. In vitro effects of hydrochloric and lactic acids on bioelectric properties of equine gastric squamous mucosa. *Equine Vet J* 2008.

51. Koefoed-Johnsen V, Ussing HH. The nature of the frog skin potential. *Acta Physiol Scand* 1958;42:298-308.

52. Argenzio RA, Meuten DJ. Short-chain fatty acids induce reversible injury of porcine colon. *Dig Dis Sci* 1991;36:1459-1468.

53. Chien WJ, Stevens CE. Coupled active transport of Na and Cl across forestomach epithelium. *Am J Physiol* 1972;223:997-1003.

54. Snow JC, Goldstein JL, Schmidt LN, et al. Rabbit esophageal cells show regulatory volume decrease: ionic basis and effect of pH. *Gastroenterology* 1993;105:102-110.

Appendix

Table A 1 Breakdown of horses by age

Horse	Age (years)	Breed
E07015	10	American Paint Horse
E07016	1	Quarter Horse
E04030	12	Mixed Breed
E07018	13	Belgian Cross
E07022	19	Thoroughbred
E07008	5	Thoroughbred
E07007	4	Thoroughbred
E07029	9	Appaloosa
E07057	26	Quarter Horse
E07044	17	Morgan
E07019	21	Thoroughbred

Table A 2 Breakdown of horses by sex

Sex	Number of Horses
Gelding	9
Mare	2
Total	**11**

Table A 3 Breakdown of horses by reason for euthanasia

Signalment	**Number of Horses**
Lameness	2
Wobbler Syndrome	1
Nasal Cyst	1
Old Age	3
Laminitis	1
No longer suitable for owners use	2
Behavioral Problems	1
Total	**11**

Table A 4 Breakdown of horses by Gastrophilus intestinais larvae present in the stomach

With larva	4
Without larvae	7
Total	**11**

Table A 5 Breakdown of horses by gastric ulcer score

Horses With Ulcers	3
Grade 1 Ulcerations	1
Grade 2 Ulcerations	1
Grade 3 Ulcerations	1
Horses Without Ulcers	8
Total	**11**

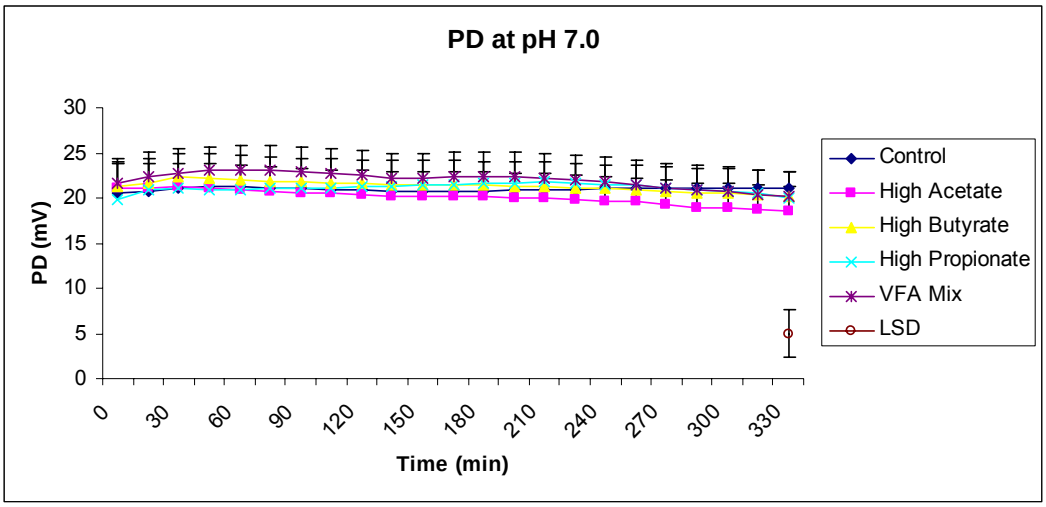

Figure A 1 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

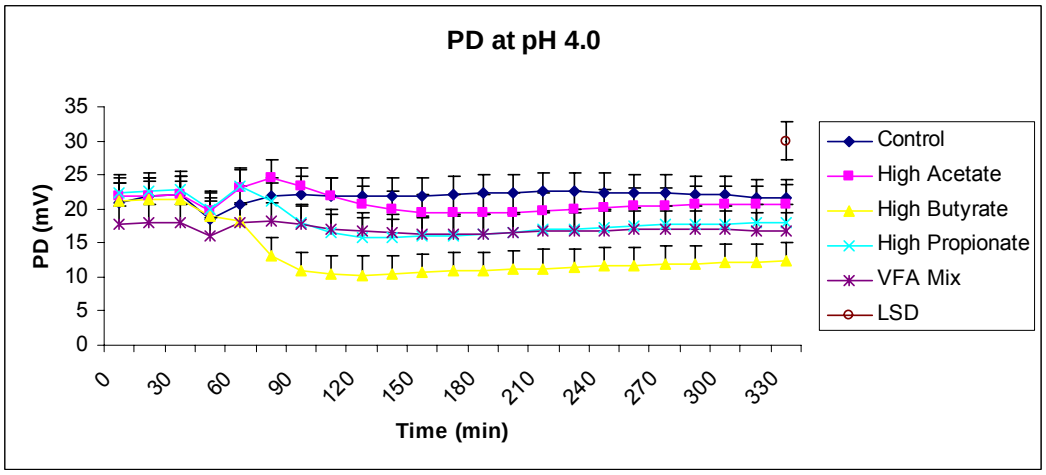

Figure A 2 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

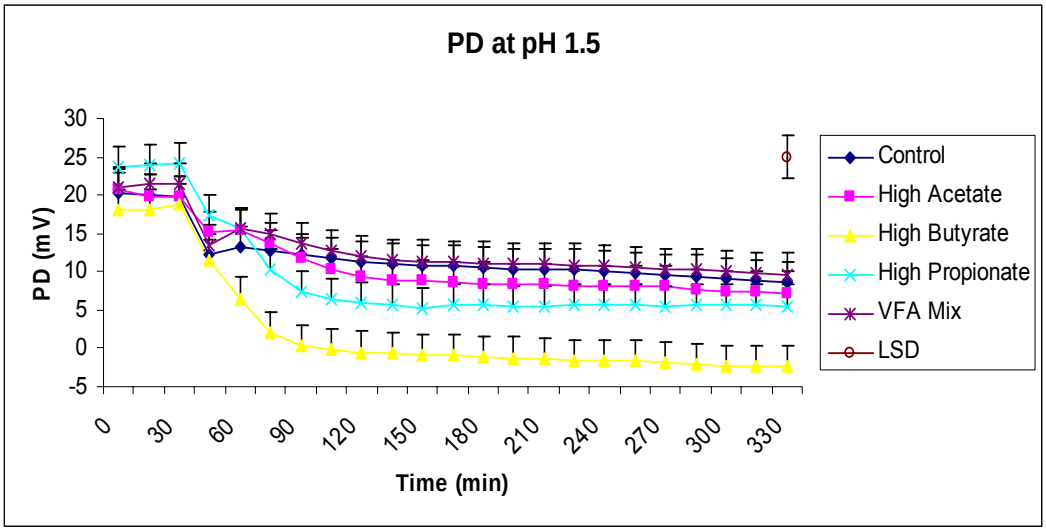

Figure A 3 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

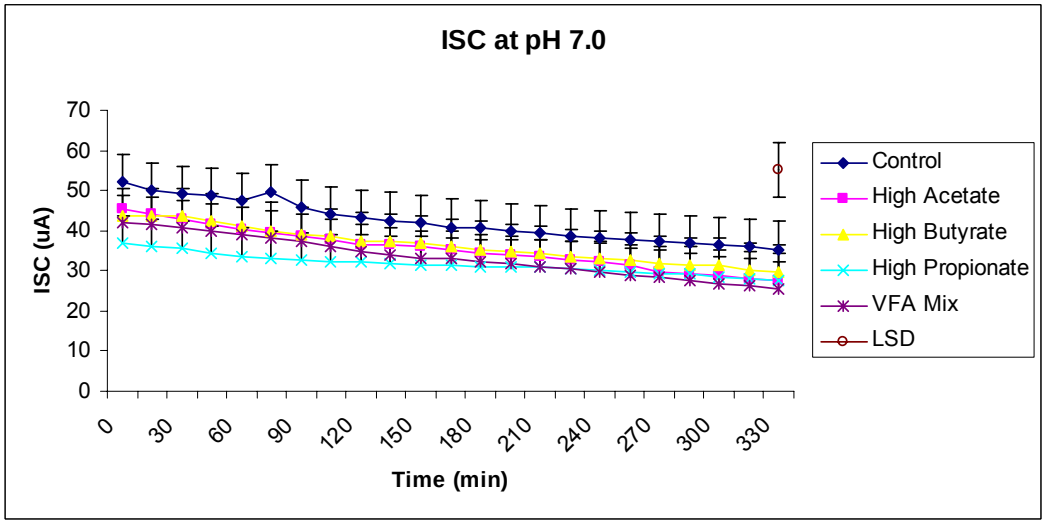

Figure A 4 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

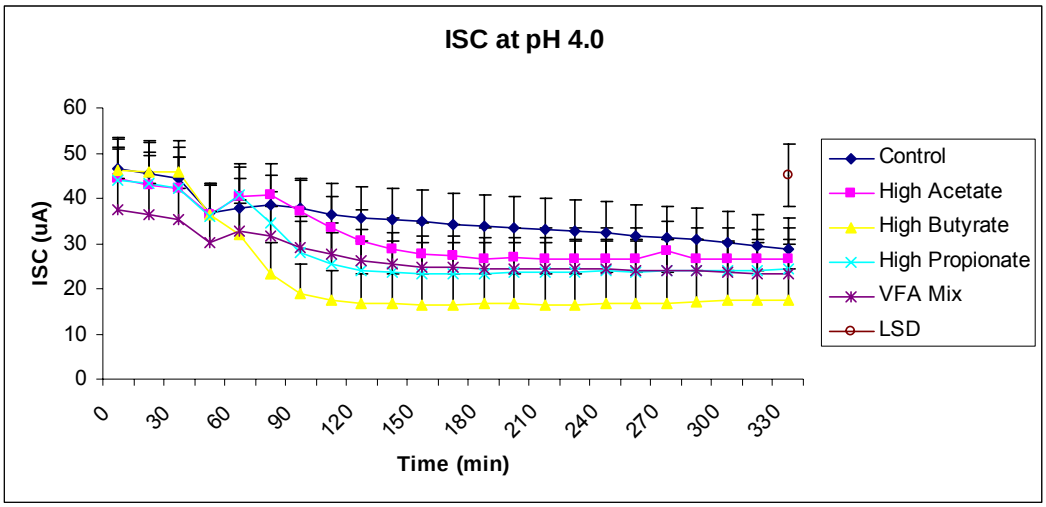

Figure A 5 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

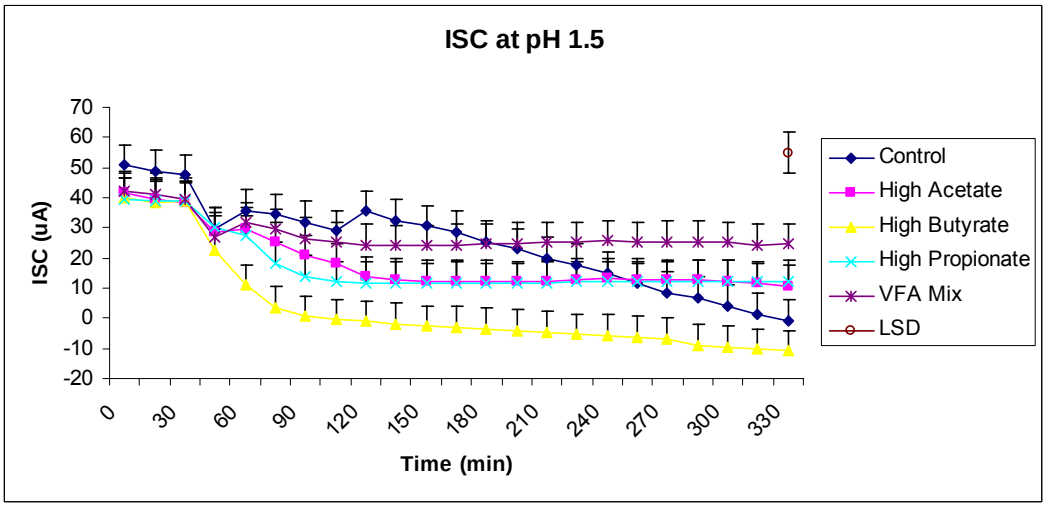

Figure A 6 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

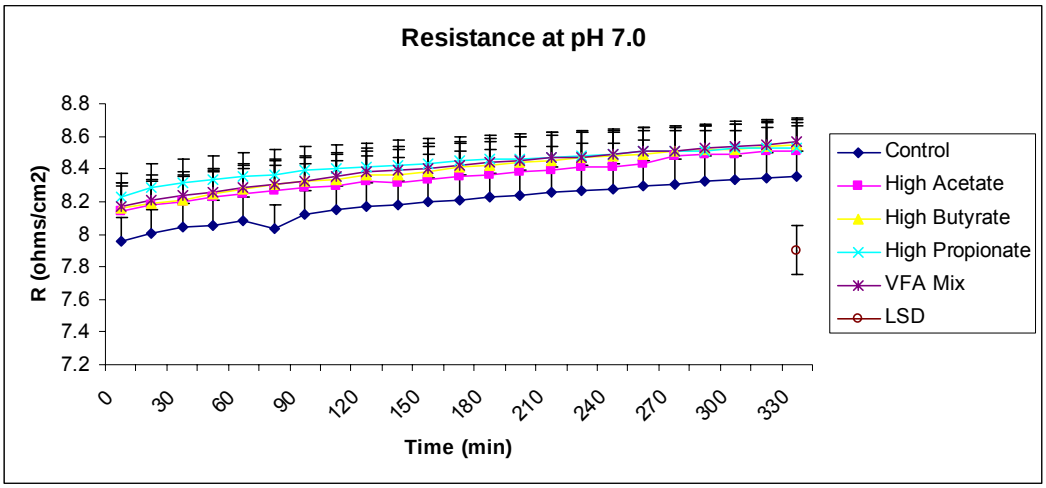

Figure A 7 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

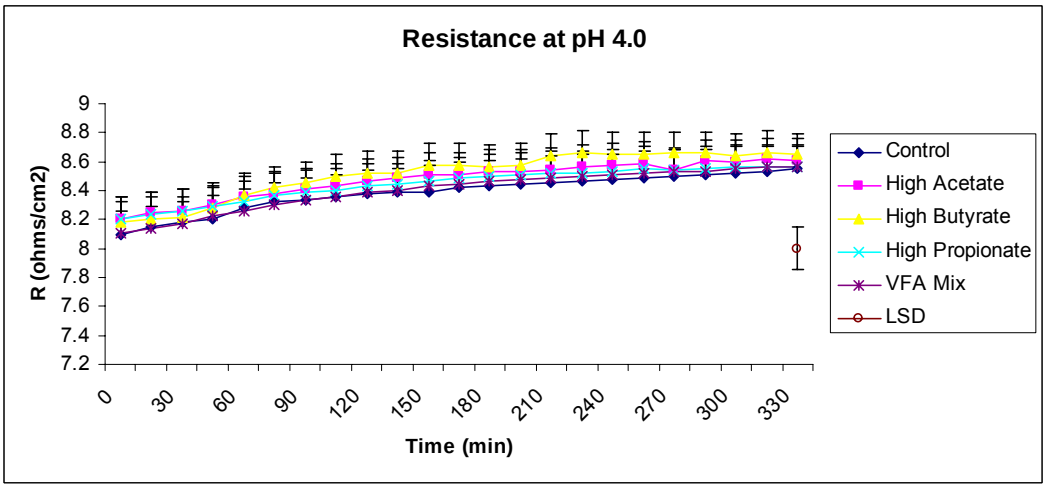

Figure A 8 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

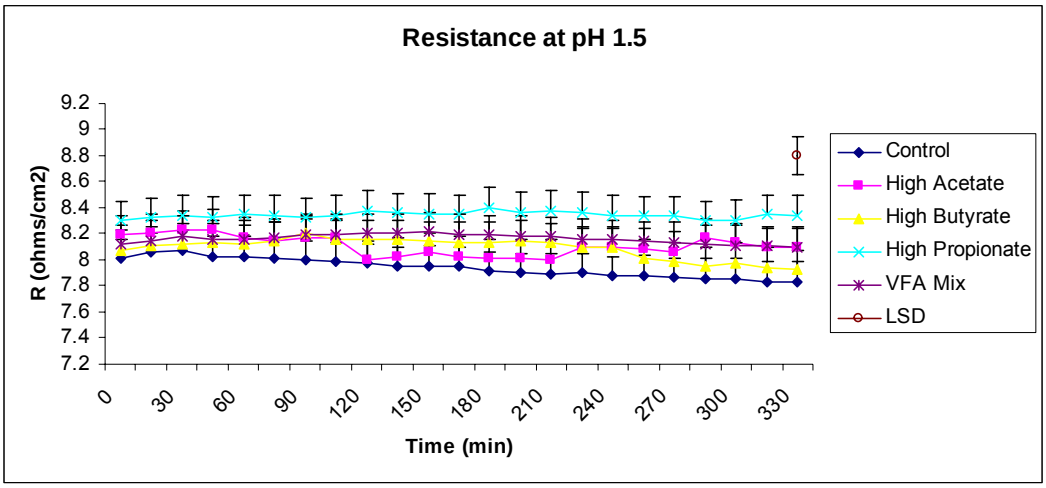

Figure A 9 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

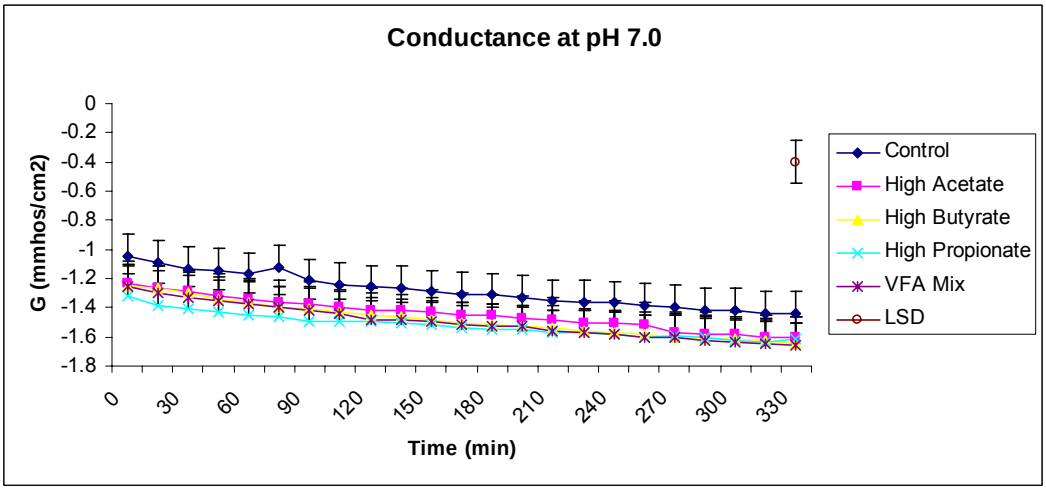

Figure A 10 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

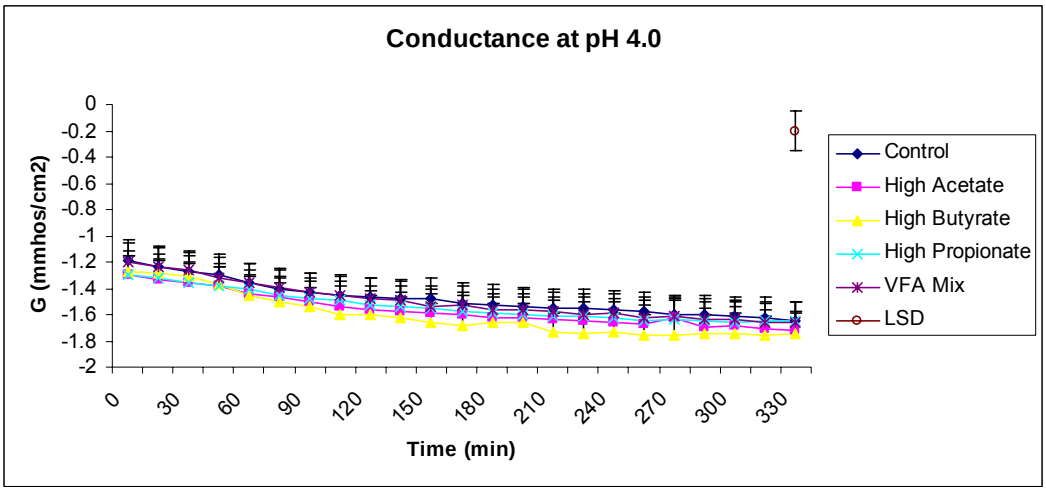

Figure A 11 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

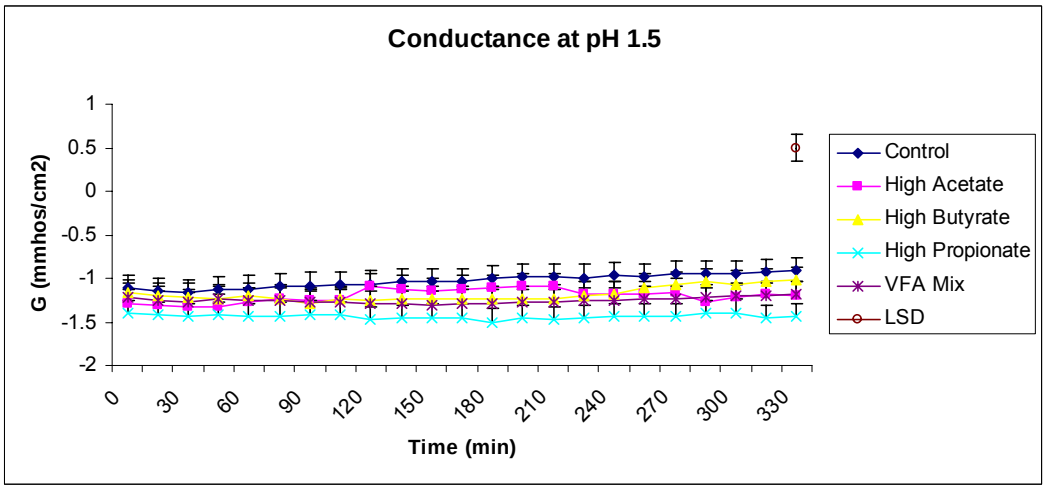

Figure A 12 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

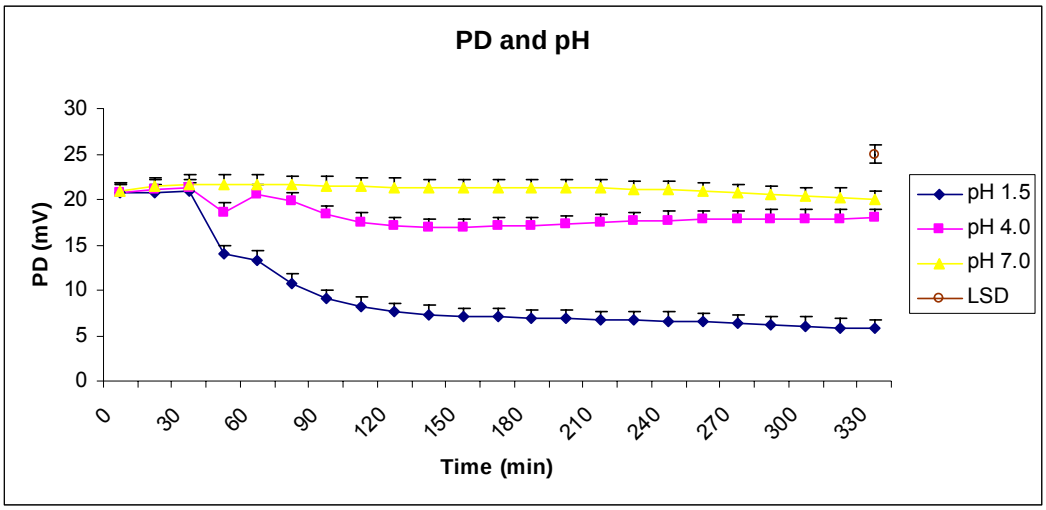

Figure A 13 Time and pH interaction. Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

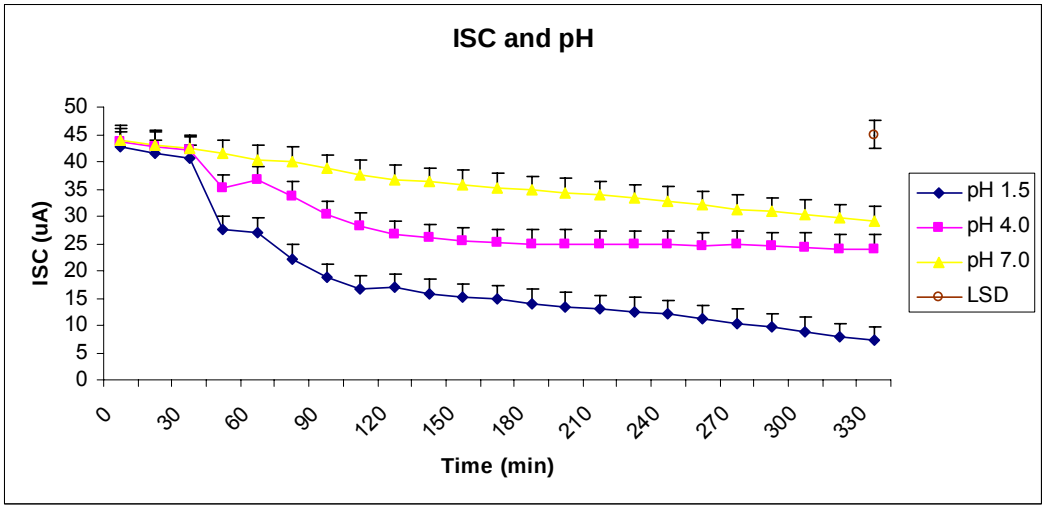

Figure A 14 Time and pH interaction. Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

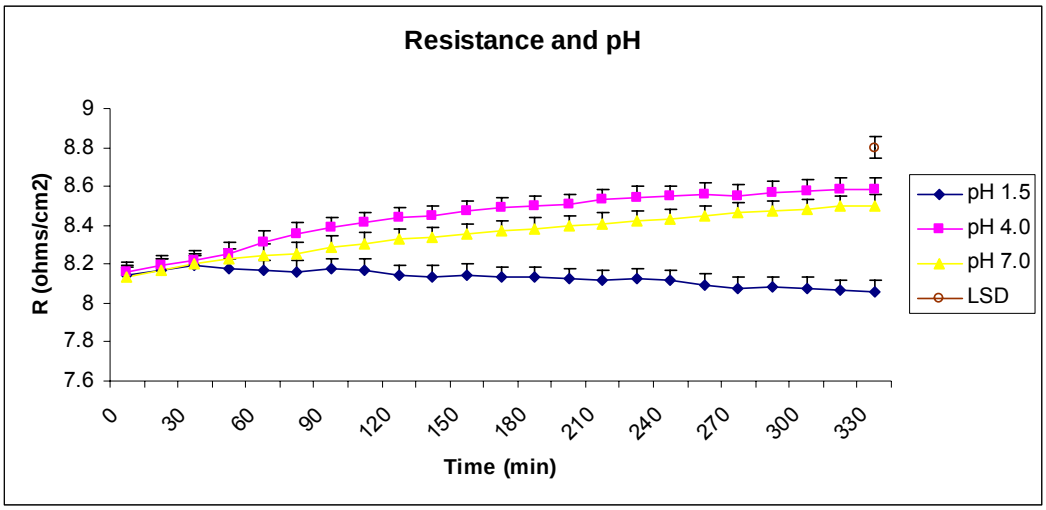

Figure A 15 Time and pH interaction. Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

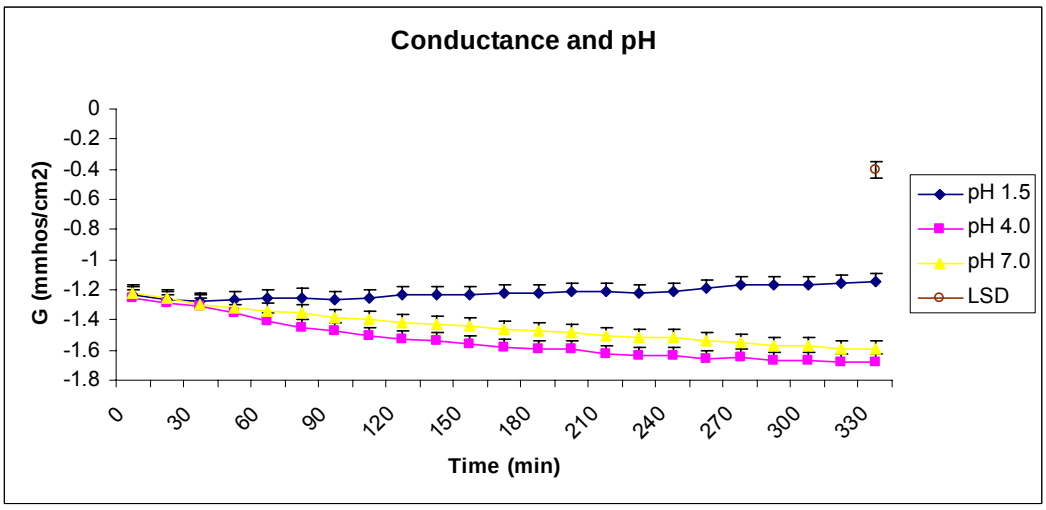

Figure A 16 Time and pH interaction. Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

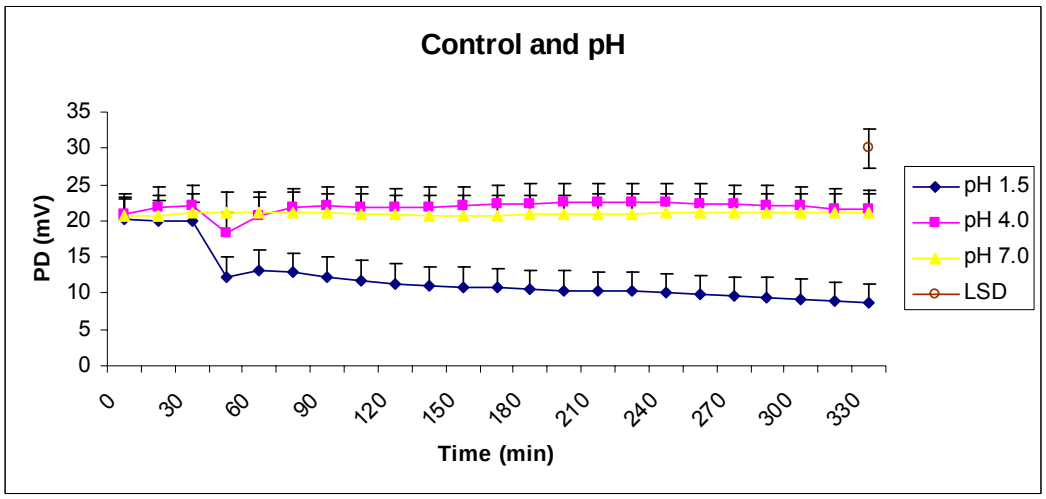

Figure A 17 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

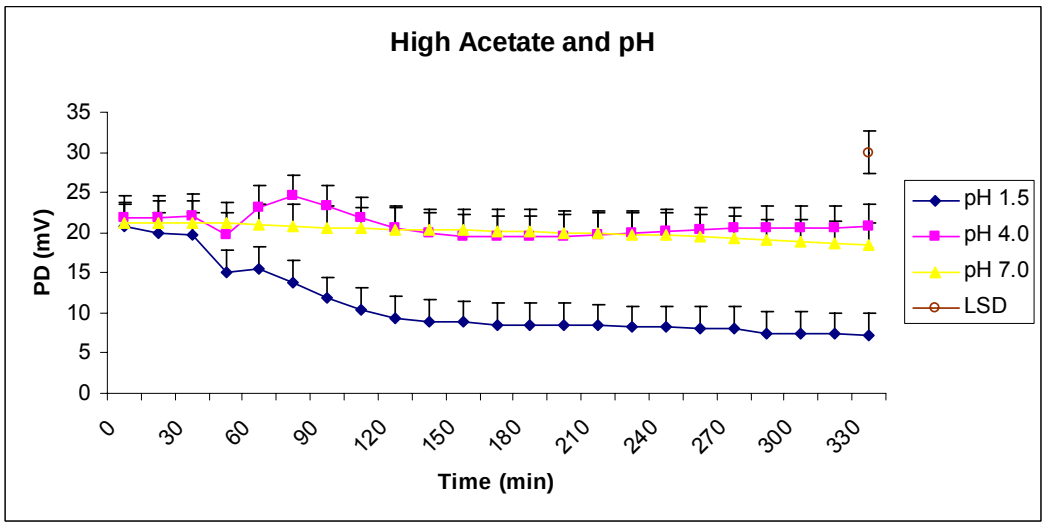

Figure A 18 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

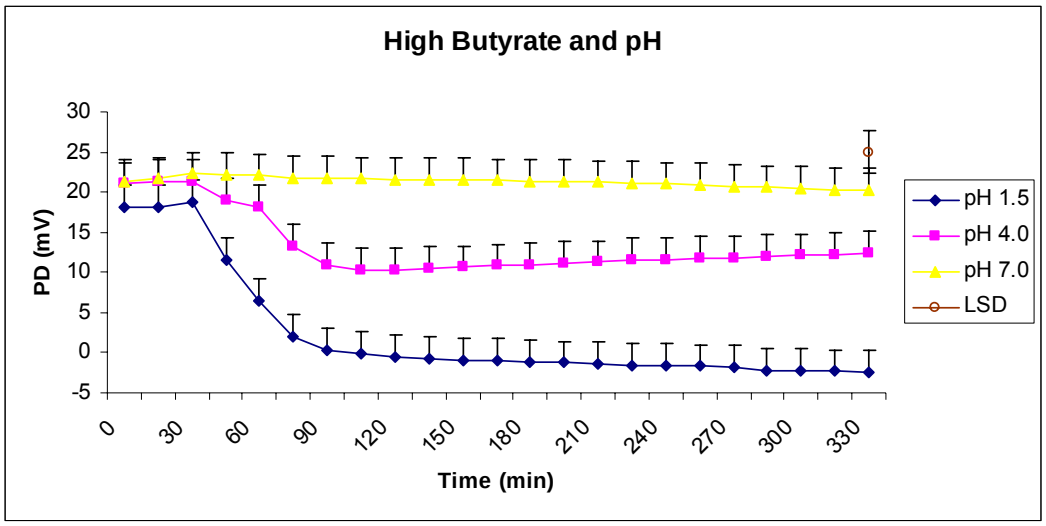

Figure A 19 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

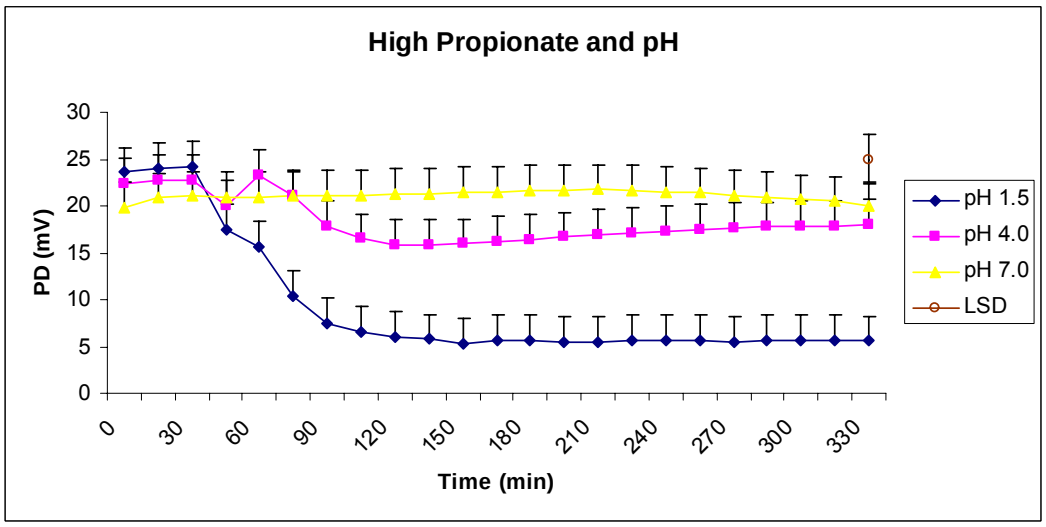

Figure A 20 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

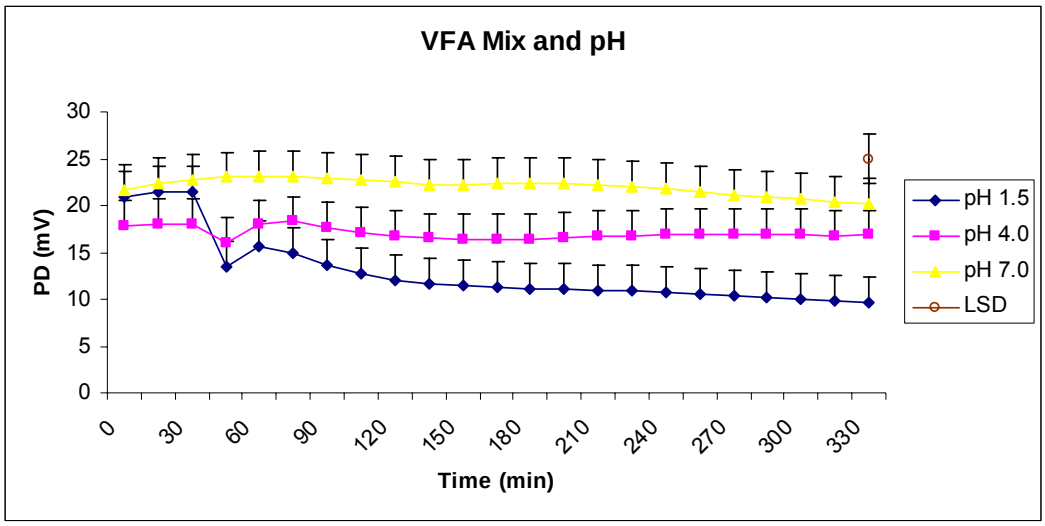

Figure A 21 Mean ± LSD values for PD in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

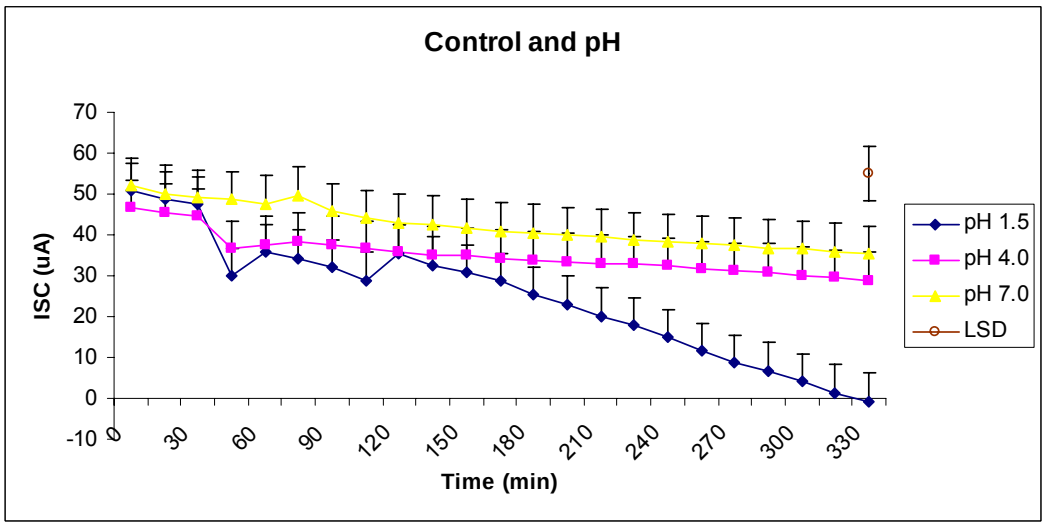

Figure A 22 Mean ± LSD values for ISC in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

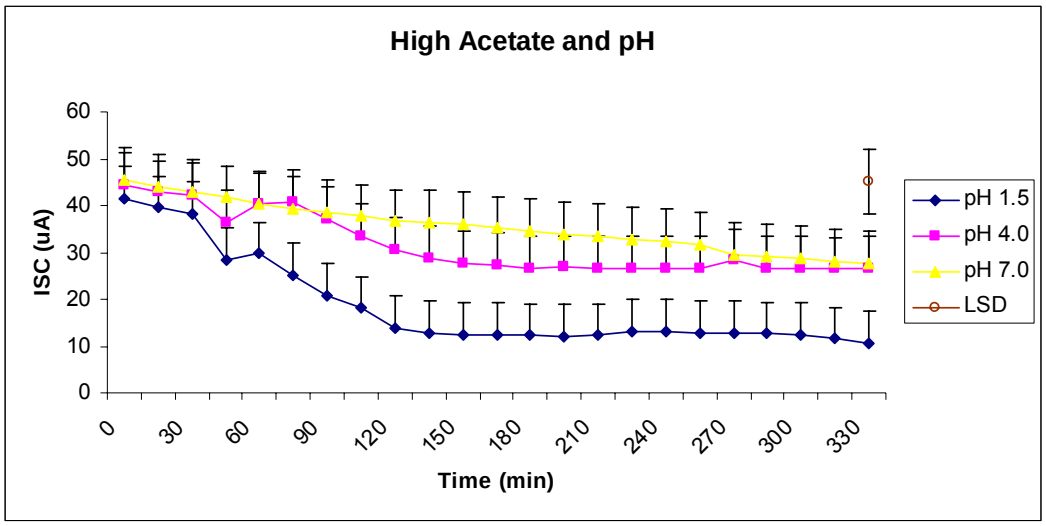

Figure A 23 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

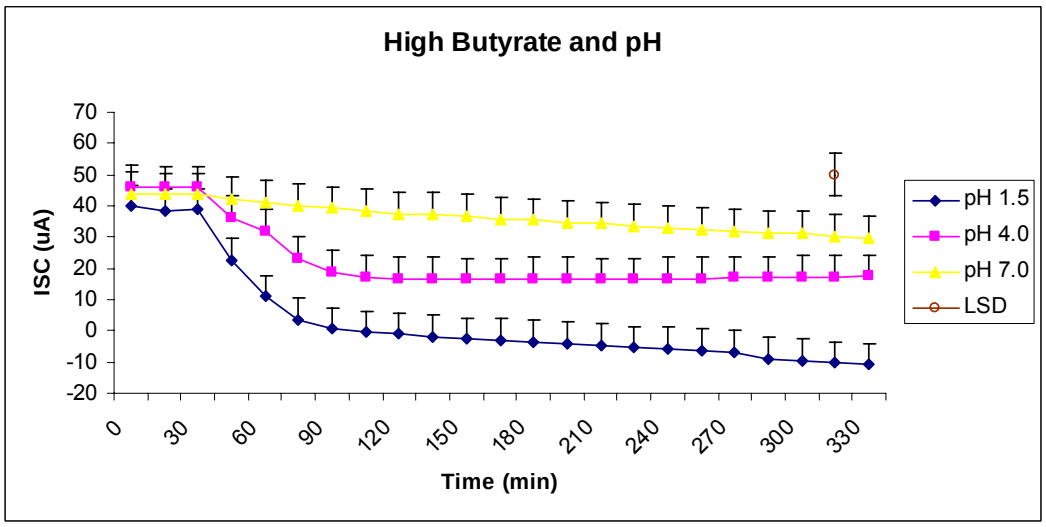

Figure A 24 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

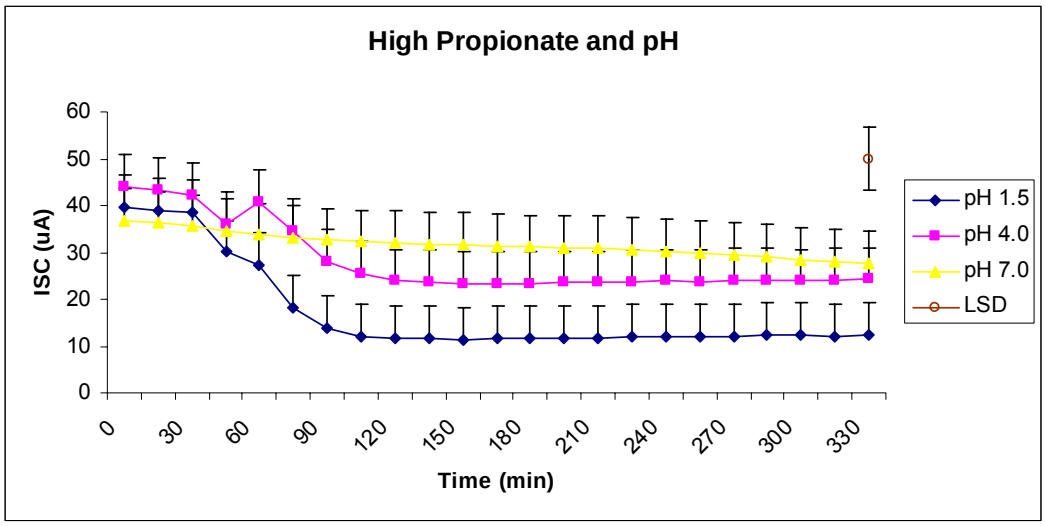

Figure A 25 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

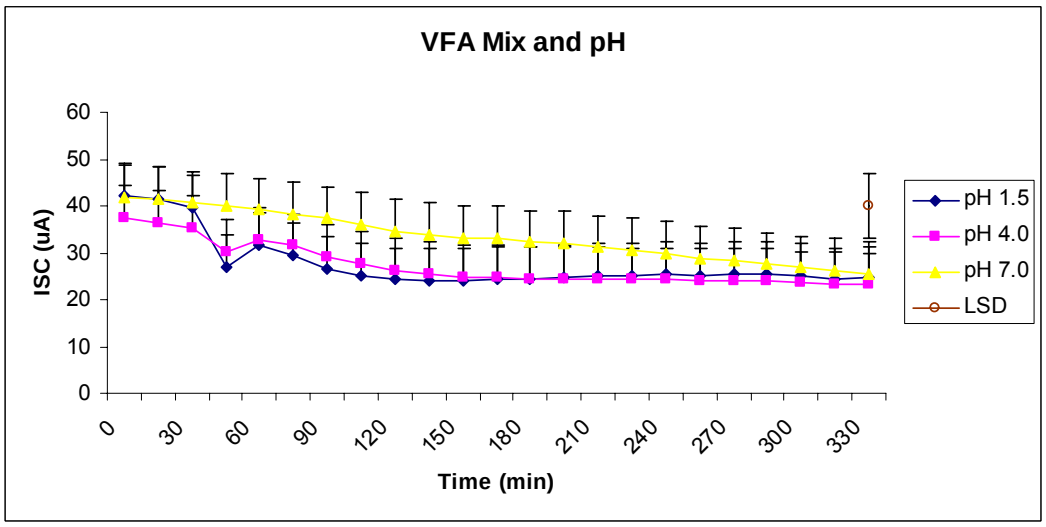

Figure A 26 Mean ± LSD values for Isc in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

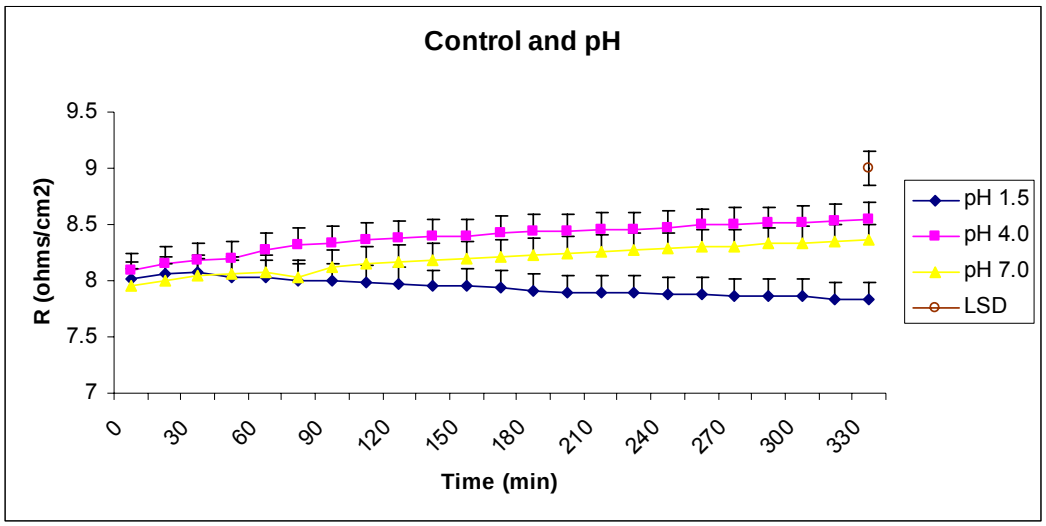

Figure A 27 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

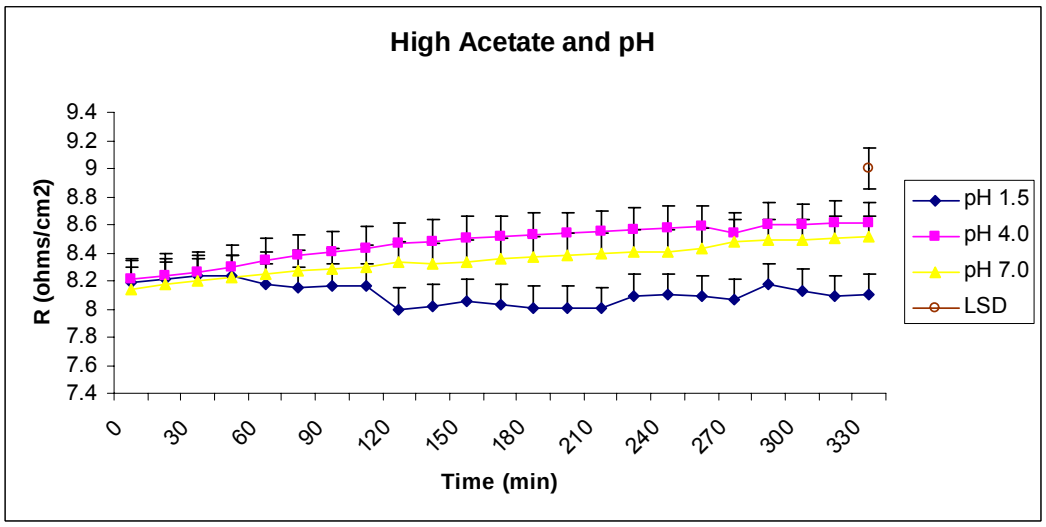

Figure A 28 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

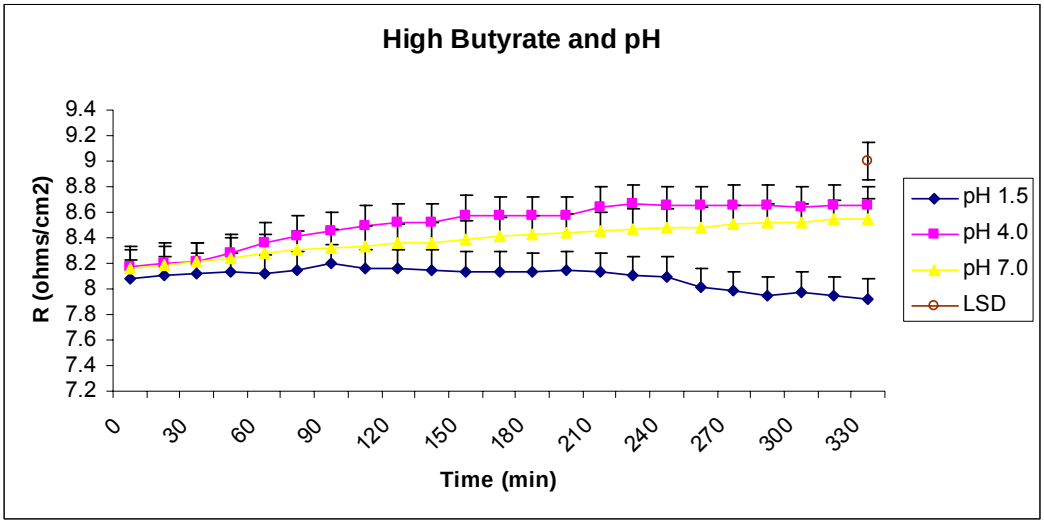

Figure A 29 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

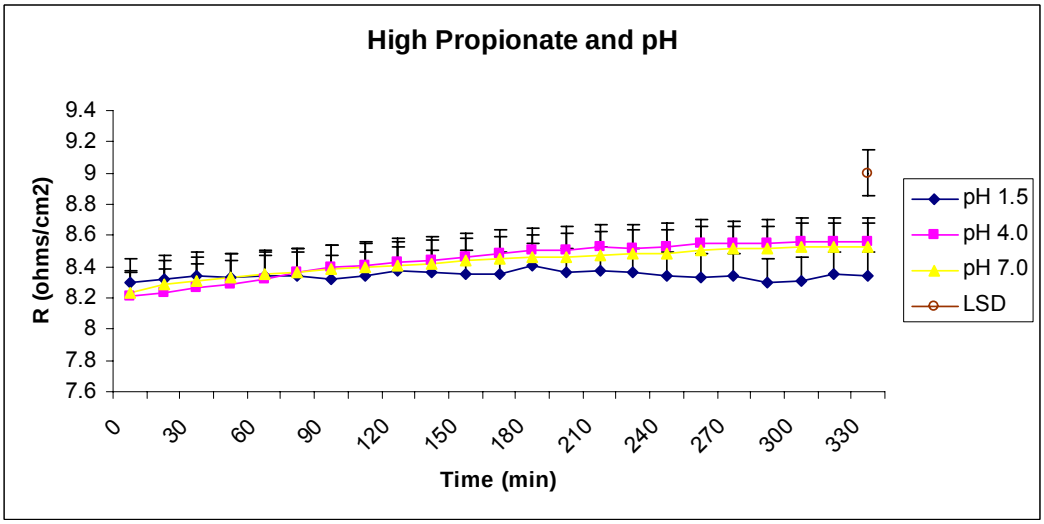

Figure A 30 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

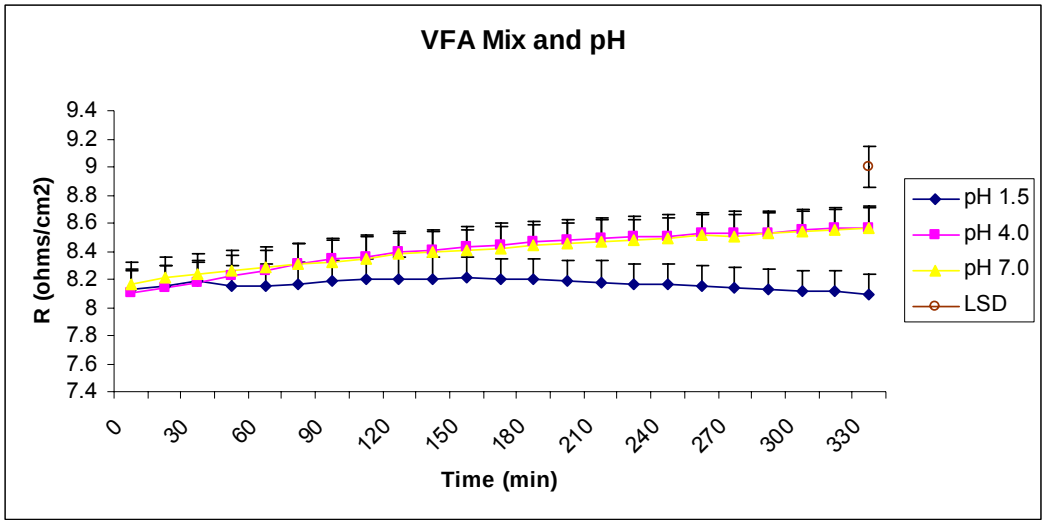

Figure A 31 Mean ± LSD values for R in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

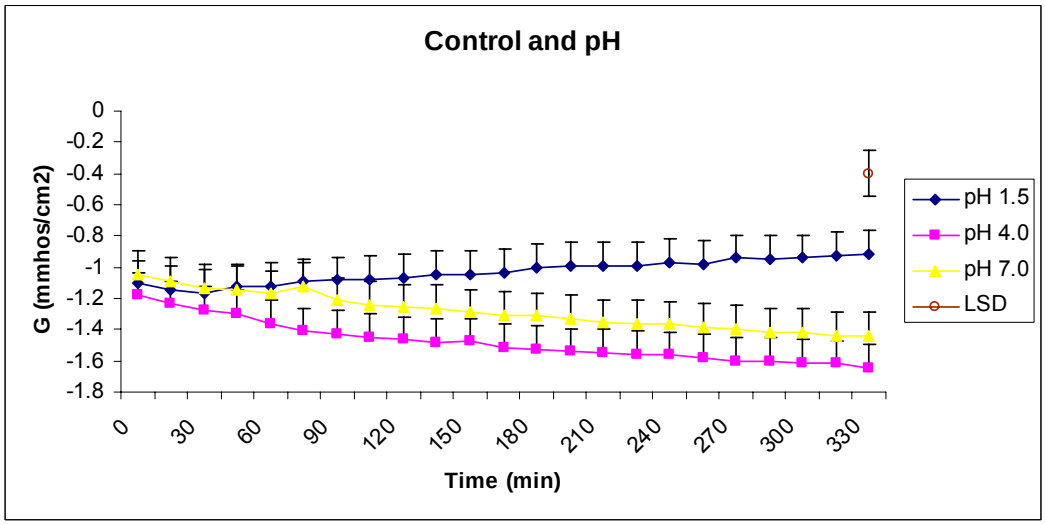

Figure A 32 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

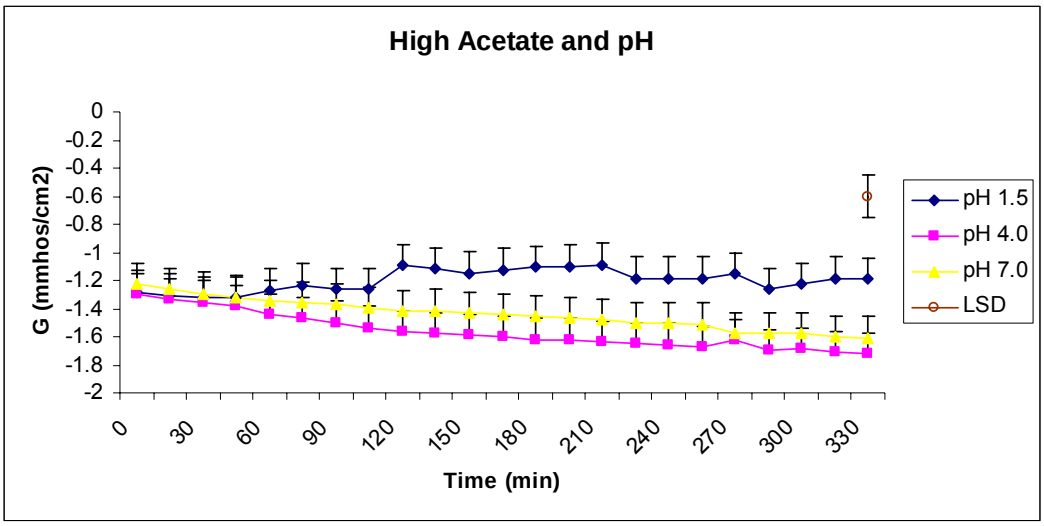

Figure A 33 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

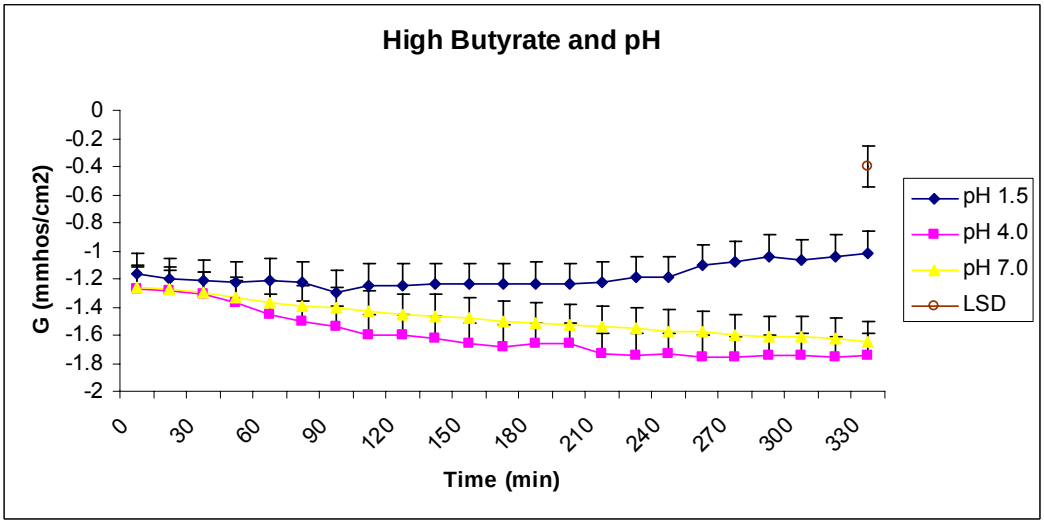

Figure A 34 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

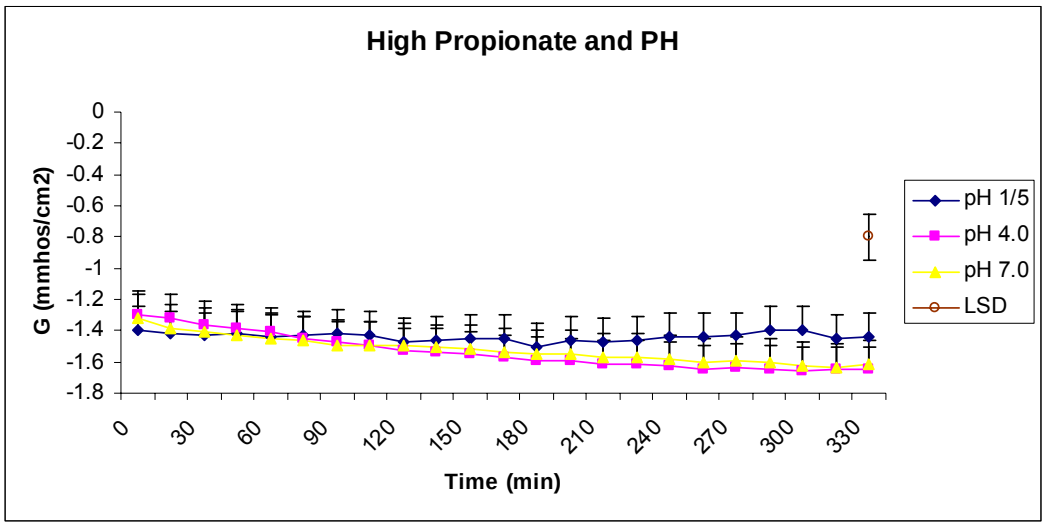

Figure A 35 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

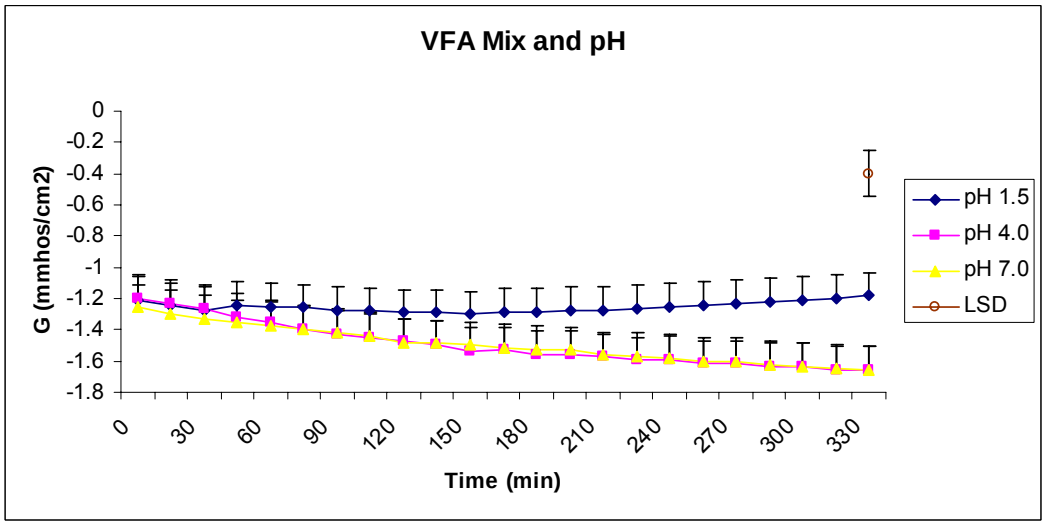

Figure A 36 Mean ± LSD values for G in NG squamous mucosa collected from each of 10 horses.
For each time point, least square mean values that differ by more than the Fisher's least significant difference (circled bars) are significantly different (P<0.05).

Table A 6. Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
Control	0	20.2135	21.0024	20.56
Control	15	20.0802	21.8802	20.78
Control	30	19.8691	22.1358	21.06
Control	45	12.2691	18.3691	21.21
Control	60	13.2135	20.558	21.22
Control	75	12.8135	21.8135	21.17
Control	90	12.2246	22.0135	21.08
Control	105	11.8135	21.8913	20.93
Control	120	11.358	21.8024	20.83
Control	135	10.9913	21.858	20.77
Control	150	10.8135	21.9691	20.77
Control	165	10.7246	22.2024	20.72
Control	180	10.5358	22.3246	20.79
Control	195	10.358	22.4469	20.87
Control	210	10.2469	22.5246	21
Control	225	10.3135	22.5024	20.95
Control	240	10.0691	22.4802	21.06
Control	255	9.8469	22.3913	21.09
Control	270	9.5358	22.2691	21.11
Control	285	9.4358	22.1913	21.1
Control	300	9.1802	22.0024	21.12
Control	315	8.8802	21.6691	21.1
Control	330	8.6691	21.6024	21.08
High Acetate	0	20.77	21.86	21.16
High Acetate	15	19.89	21.84	21.18
High Acetate	30	19.82	22.02	21.2
High Acetate	45	15.09	19.76	21.14
High Acetate	60	15.51	23.14	20.93
High Acetate	75	13.81	24.52	20.8
High Acetate	90	11.78	23.24	20.62
High Acetate	105	10.35	21.76	20.47
High Acetate	120	9.37	20.62	20.37
High Acetate	135	8.87	19.87	20.27
High Acetate	150	8.81	19.52	20.26
High Acetate	165	8.55	19.41	20.15
High Acetate	180	8.47	19.41	20.12
High Acetate	195	8.45	19.56	20.03
High Acetate	210	8.43	19.73	19.99
High Acetate	225	8.19	19.96	19.83
High Acetate	240	8.21	20.17	19.72
High Acetate	255	8.16	20.32	19.57
High Acetate	270	8.06	20.48	19.35
High Acetate	285	7.53	20.58	19
High Acetate	300	7.49	20.59	18.95
High Acetate	315	7.35	20.67	18.74
High Acetate	330	7.25	20.76	18.56

Table A 7 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
High Butyrate	0	18.1797	21.03	21.258
High Butyrate	15	18.1464	21.33	21.658
High Butyrate	30	18.7575	21.39	22.2802
High Butyrate	45	11.5464	18.93	22.2024
High Butyrate	60	6.5242	18.19	22.0691
High Butyrate	75	1.9686	13.22	21.8246
High Butyrate	90	0.3908	10.97	21.7469
High Butyrate	105	-0.1092	10.36	21.6246
High Butyrate	120	-0.5092	10.32	21.558
High Butyrate	135	-0.7314	10.47	21.5135
High Butyrate	150	-0.9758	10.64	21.5135
High Butyrate	165	-0.9758	10.86	21.4135
High Butyrate	180	-1.1203	11.01	21.3691
High Butyrate	195	-1.2425	11.15	21.3024
High Butyrate	210	-1.3981	11.28	21.2135
High Butyrate	225	-1.5425	11.5	21.0802
High Butyrate	240	-1.6203	11.61	21.0246
High Butyrate	255	-1.6981	11.73	20.858
High Butyrate	270	-1.7758	11.86	20.7469
High Butyrate	285	-2.187	11.98	20.5691
High Butyrate	300	-2.2758	12.09	20.5024
High Butyrate	315	-2.3314	12.21	20.3246
High Butyrate	330	-2.3981	12.4	20.2024
High Propionate	0	23.5691	22.3024	19.9
High Propionate	15	23.9802	22.6802	20.82
High Propionate	30	24.1913	22.7691	21.02
High Propionate	45	17.4469	19.958	21
High Propionate	60	15.6691	23.2802	20.98
High Propionate	75	10.4024	21.0691	21.01
High Propionate	90	7.4469	17.9024	21.1
High Propionate	105	6.5024	16.458	21.11
High Propionate	120	6.0024	15.9024	21.24
High Propionate	135	5.7469	15.8024	21.32
High Propionate	150	5.258	15.9246	21.45
High Propionate	165	5.6024	16.158	21.52
High Propionate	180	5.5802	16.4024	21.58
High Propionate	195	5.5358	16.6469	21.63
High Propionate	210	5.4802	16.9024	21.74
High Propionate	225	5.5913	17.1246	21.63
High Propionate	240	5.6024	17.3024	21.49
High Propionate	255	5.5913	17.4913	21.37
High Propionate	270	5.5358	17.6246	21.14
High Propionate	285	5.658	17.7358	20.88
High Propionate	300	5.6469	17.8358	20.66
High Propionate	315	5.5913	17.8802	20.46
High Propionate	330	5.5469	18.0135	19.93

Table A 8Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
VFA Mix	0	20.958	17.758	21.6135
VFA Mix	15	21.5024	18.0246	22.4246
VFA Mix	30	21.5358	18.058	22.758
VFA Mix	45	13.4246	16.0691	23.0246
VFA Mix	60	15.6024	17.9135	23.1246
VFA Mix	75	14.9913	18.2802	23.0469
VFA Mix	90	13.6913	17.7024	22.9802
VFA Mix	105	12.6913	17.1135	22.758
VFA Mix	120	12.0135	16.7024	22.4913
VFA Mix	135	11.6024	16.4691	22.2135
VFA Mix	150	11.3913	16.358	22.158
VFA Mix	165	11.2469	16.3913	22.3802
VFA Mix	180	11.1469	16.3913	22.3469
VFA Mix	195	11.0469	16.5802	22.3469
VFA Mix	210	10.9469	16.6913	22.2469
VFA Mix	225	10.9024	16.8024	22.0358
VFA Mix	240	10.7246	16.8802	21.8246
VFA Mix	255	10.5469	16.9469	21.5024
VFA Mix	270	10.3246	16.9469	21.1802
VFA Mix	285	10.2691	16.958	20.9135
VFA Mix	300	10.0691	16.9024	20.6802
VFA Mix	315	9.7802	16.7913	20.4024
VFA Mix	330	9.6802	16.8358	20.158

Table A 9 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
Control	0	50.7458	46.6	52
Control	15	48.7458	45.6	50.1
Control	30	47.4125	44.5	49.1
Control	45	29.8569	36.6	48.6
Control	60	35.7458	37.7	47.6
Control	75	34.3014	38.4	49.7
Control	90	31.968	37.7	45.7
Control	105	28.8569	36.5	44.2
Control	120	35.5236	35.8	43.1
Control	135	32.6347	35.2	42.6
Control	150	30.6347	35	41.8
Control	165	28.6347	34.3	40.9
Control	180	25.4125	33.9	40.6
Control	195	22.968	33.5	40
Control	210	20.0791	33.1	39.4
Control	225	17.8569	32.8	38.7
Control	240	14.968	32.3	38.3
Control	255	11.5236	31.7	37.8
Control	270	8.6347	31.2	37.3
Control	285	6.8569	30.9	36.8
Control	300	4.0791	30.2	36.5
Control	315	1.4125	29.4	35.9
Control	330	-0.6986	28.9	35.4
High Acetate	0	41.6	44.3	45.6
High Acetate	15	39.5	42.8	44.1
High Acetate	30	38.2	42.1	42.9
High Acetate	45	28.3	36.3	41.7
High Acetate	60	29.7	40.2	40.4
High Acetate	75	25.2	40.9	39.3
High Acetate	90	20.8	37.2	38.6
High Acetate	105	18.03	33.5	37.7
High Acetate	120	13.8	30.7	36.6
High Acetate	135	12.9	28.9	36.4
High Acetate	150	12.3	27.6	35.9
High Acetate	165	12.4	27.3	35.1
High Acetate	180	12.2	26.7	34.5
High Acetate	195	12.1	26.8	33.9
High Acetate	210	12.2	26.7	33.4
High Acetate	225	13	26.5	32.7
High Acetate	240	13.2	26.5	32.4
High Acetate	255	12.8	26.5	31.6
High Acetate	270	12.7	28.2	29.5
High Acetate	285	12.6	26.5	29.1
High Acetate	300	12.4	26.7	28.9
High Acetate	315	11.5	26.4	28.1
High Acetate	330	10.6	26.6	27.6

Table A 10 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
High Butyrate	0	39.7983	46.1	43.8489
High Butyrate	15	38.5761	45.8	43.7937
High Butyrate	30	38.7983	45.8	43.7378
High Butyrate	45	22.6872	36.2	42.4044
High Butyrate	60	11.0205	32	41.2933
High Butyrate	75	3.5761	23.2	40.0711
High Butyrate	90	0.5761	18.8	39.1822
High Butyrate	105	-0.5351	17.3	38.4044
High Butyrate	120	-1.0906	16.6	37.5156
High Butyrate	135	-1.9795	16.6	37.4044
High Butyrate	150	-2.6462	16.3	36.7378
High Butyrate	165	-2.9795	16.4	35.8489
High Butyrate	180	-3.5351	16.6	35.4044
High Butyrate	195	-4.0906	16.6	34.7378
High Butyrate	210	-4.6462	16.5	34.2933
High Butyrate	225	-5.4239	16.5	33.5156
High Butyrate	240	-5.7573	16.7	33.0711
High Butyrate	255	-6.3128	16.8	32.6267
High Butyrate	270	-6.8684	16.9	31.96
High Butyrate	285	-8.9795	17	31.2933
High Butyrate	300	-9.6462	17.3	31.2933
High Butyrate	315	-10.2017	17.3	30.1822
High Butyrate	330	-10.8684	17.6	29.8489
High Propionate	0	39.669	43.96	36.9
High Propionate	15	39.044	43.4044	36.2
High Propionate	30	38.669	42.2933	35.5
High Propionate	45	30.044	36.0711	34.5
High Propionate	60	27.294	40.8489	33.7
High Propionate	75	18.294	34.5156	33.2
High Propionate	90	13.919	28.0711	32.6
High Propionate	105	12.169	25.4044	32.2
High Propionate	120	11.669	23.8489	32.1
High Propionate	135	11.644	23.6267	31.7
High Propionate	150	11.419	23.4044	31.6
High Propionate	165	11.669	23.2933	31.3
High Propionate	180	11.544	23.1822	31.1
High Propionate	195	11.544	23.5156	31
High Propionate	210	11.669	23.5156	31
High Propionate	225	12.044	23.7378	30.6
High Propionate	240	12.044	23.8489	30.3
High Propionate	255	12.169	23.7378	29.8
High Propionate	270	12.044	24.0711	29.4
High Propionate	285	12.294	23.96	29.1
High Propionate	300	12.419	23.8489	28.5
High Propionate	315	12.169	23.96	28.1
High Propionate	330	12.294	24.1822	27.6

Table A 11 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
VFA Mix	0	42.1316	37.5	42
VFA Mix	15	41.3538	36.5	41.5
VFA Mix	30	39.6872	35.3	40.6
VFA Mix	45	27.0205	30.3	40
VFA Mix	60	31.7983	32.7	39.1
VFA Mix	75	29.5761	31.5	38.1
VFA Mix	90	26.5761	29.2	37.3
VFA Mix	105	25.0205	27.6	36
VFA Mix	120	24.2427	26.2	34.7
VFA Mix	135	23.9094	25.5	33.9
VFA Mix	150	24.0205	24.9	33.2
VFA Mix	165	24.3538	24.7	33
VFA Mix	180	24.4649	24.4	32.2
VFA Mix	195	24.7983	24.4	31.9
VFA Mix	210	25.2427	24.3	31.1
VFA Mix	225	25.1316	24.2	30.5
VFA Mix	240	25.5761	24.2	29.9
VFA Mix	255	25.2427	24	28.9
VFA Mix	270	25.4649	23.9	28.3
VFA Mix	285	25.4649	23.9	27.5
VFA Mix	300	25.0205	23.5	26.8
VFA Mix	315	24.2427	23.3	26.2
VFA Mix	330	24.5761	23.1	25.5

Table A 12 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on short logresistance (R) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
Control	0	8.0134	8.09	7.9561
Control	15	8.0568	8.1465	8.0013
Control	30	8.0753	8.1814	8.0412
Control	45	8.0303	8.1996	8.0553
Control	60	8.0289	8.2754	8.0807
Control	75	8.0067	8.3227	8.0306
Control	90	7.9965	8.3355	8.119
Control	105	7.9856	8.3606	8.147
Control	120	7.9754	8.3746	8.1687
Control	135	7.9478	8.3882	8.1776
Control	150	7.9563	8.3899	8.1964
Control	165	7.946	8.4205	8.2132
Control	180	7.9159	8.4352	8.2232
Control	195	7.9	8.4456	8.2392
Control	210	7.8928	8.4586	8.2589
Control	225	7.8973	8.4616	8.2685
Control	240	7.88	8.4753	8.2812
Control	255	7.8817	8.4927	8.298
Control	270	7.8619	8.5014	8.3081
Control	285	7.8581	8.5077	8.3269
Control	300	7.8582	8.5226	8.3318
Control	315	7.835	8.5323	8.3455
Control	330	7.8359	8.5485	8.3571
High Acetate	0	8.1914	8.2082	8.1433
High Acetate	15	8.2071	8.2421	8.1784
High Acetate	30	8.2308	8.2615	8.2032
High Acetate	45	8.2347	8.3012	8.2295
High Acetate	60	8.172	8.3511	8.2516
High Acetate	75	8.1466	8.3814	8.271
High Acetate	90	8.1656	8.4065	8.2853
High Acetate	105	8.1656	8.4353	8.3
High Acetate	120	7.9998	8.4651	8.3274
High Acetate	135	8.0213	8.482	8.3199
High Acetate	150	8.06	8.5078	8.3349
High Acetate	165	8.0275	8.5098	8.3547
High Acetate	180	8.0113	8.5316	8.3669
High Acetate	195	8.0109	8.535	8.3839
High Acetate	210	8.0022	8.5463	8.3911
High Acetate	225	8.0949	8.5677	8.408
High Acetate	240	8.0973	8.5777	8.4085
High Acetate	255	8.0885	8.5859	8.4285
High Acetate	270	8.0655	8.5378	8.4812
High Acetate	285	8.1709	8.602	8.4854
High Acetate	300	8.129	8.5964	8.4873
High Acetate	315	8.092	8.6146	8.5068
High Acetate	330	8.1019	8.6103	8.5117

Table A 13 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logresistance (R) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
High Butyrate	0	8.0771	8.1767	8.162
High Butyrate	15	8.1084	8.2036	8.1847
High Butyrate	30	8.1262	8.2094	8.2129
High Butyrate	45	8.1334	8.2752	8.2446
High Butyrate	60	8.1226	8.3662	8.2748
High Butyrate	75	8.1405	8.4181	8.301
High Butyrate	90	8.2027	8.4507	8.322
High Butyrate	105	8.154	8.4989	8.3372
High Butyrate	120	8.1547	8.518	8.3611
High Butyrate	135	8.1518	8.5203	8.3662
High Butyrate	150	8.1399	8.5775	8.3856
High Butyrate	165	8.1386	8.5752	8.4103
High Butyrate	180	8.1359	8.5671	8.4215
High Butyrate	195	8.1471	8.5723	8.4417
High Butyrate	210	8.131	8.6439	8.4524
High Butyrate	225	8.1025	8.6612	8.4714
High Butyrate	240	8.0993	8.6525	8.4792
High Butyrate	255	8.013	8.6534	8.4857
High Butyrate	270	7.9864	8.6576	8.5052
High Butyrate	285	7.9477	8.6573	8.518
High Butyrate	300	7.978	8.6453	8.5193
High Butyrate	315	7.9423	8.6591	8.5425
High Butyrate	330	7.9255	8.6479	8.5501
High Propionate	0	8.3028	8.2084	8.2273
High Propionate	15	8.3246	8.2357	8.2854
High Propionate	30	8.3428	8.263	8.3116
High Propionate	45	8.3307	8.2879	8.3331
High Propionate	60	8.3464	8.3203	8.3511
High Propionate	75	8.3423	8.3664	8.3666
High Propionate	90	8.3218	8.3921	8.388
High Propionate	105	8.3444	8.4047	8.398
High Propionate	120	8.3796	8.4341	8.4083
High Propionate	135	8.3594	8.4403	8.4231
High Propionate	150	8.3549	8.46	8.4349
High Propionate	165	8.3476	8.4842	8.4479
High Propionate	180	8.4034	8.5021	8.4578
High Propionate	195	8.3662	8.5071	8.4638
High Propionate	210	8.3773	8.5227	8.4726
High Propionate	225	8.368	8.5214	8.4818
High Propionate	240	8.3444	8.5304	8.4885
High Propionate	255	8.3344	8.5522	8.5043
High Propionate	270	8.3388	8.5455	8.5113
High Propionate	285	8.3011	8.5524	8.5116
High Propionate	300	8.3064	8.5651	8.5279
High Propionate	315	8.3484	8.56	8.5327
High Propionate	330	8.3445	8.5599	8.5305

Table A 14 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logresistance (R) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
VFA Mix	0	8.1215	8.1072	8.1663
VFA Mix	15	8.1509	8.1414	8.2089
VFA Mix	30	8.1855	8.1754	8.2367
VFA Mix	45	8.1522	8.2226	8.2571
VFA Mix	60	8.1543	8.261	8.2856
VFA Mix	75	8.1692	8.3056	8.3081
VFA Mix	90	8.1883	8.34	8.3267
VFA Mix	105	8.1956	8.3605	8.3513
VFA Mix	120	8.2032	8.3927	8.3794
VFA Mix	135	8.2026	8.4045	8.3897
VFA Mix	150	8.2124	8.4292	8.4062
VFA Mix	165	8.1957	8.4456	8.421
VFA Mix	180	8.1952	8.4611	8.4415
VFA Mix	195	8.1872	8.4732	8.4501
VFA Mix	210	8.1791	8.4875	8.471
VFA Mix	225	8.1633	8.4996	8.4743
VFA Mix	240	8.1586	8.5056	8.4914
VFA Mix	255	8.1461	8.5237	8.5092
VFA Mix	270	8.1352	8.5293	8.5077
VFA Mix	285	8.1223	8.5327	8.5274
VFA Mix	300	8.1124	8.5527	8.5406
VFA Mix	315	8.11	8.5616	8.5514
VFA Mix	330	8.0924	8.5689	8.5647

Table A 15 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
Control	0	-1.1055	-1.1832	-1.0463
Control	15	-1.1424	-1.2365	-1.0859
Control	30	-1.1668	-1.2723	-1.1367
Control	45	-1.1271	-1.2949	-1.1461
Control	60	-1.1211	-1.3598	-1.1727
Control	75	-1.0951	-1.4104	-1.1207
Control	90	-1.0841	-1.4292	-1.2163
Control	105	-1.0771	-1.4531	-1.2397
Control	120	-1.067	-1.4666	-1.2579
Control	135	-1.044	-1.4808	-1.2623
Control	150	-1.0435	-1.4766	-1.2912
Control	165	-1.0375	-1.5113	-1.3086
Control	180	-1.0028	-1.5248	-1.3141
Control	195	-0.9904	-1.5418	-1.33
Control	210	-0.9892	-1.5515	-1.3573
Control	225	-0.9918	-1.5549	-1.3604
Control	240	-0.9726	-1.5642	-1.3674
Control	255	-0.9809	-1.5795	-1.3817
Control	270	-0.9434	-1.5996	-1.395
Control	285	-0.9457	-1.5996	-1.4201
Control	300	-0.9423	-1.6125	-1.4209
Control	315	-0.9299	-1.6185	-1.4368
Control	330	-0.9169	-1.6485	-1.4421
High Acetate	0	-1.2821	-1.3006	-1.2276
High Acetate	15	-1.3044	-1.3331	-1.2618
High Acetate	30	-1.3201	-1.3552	-1.292
High Acetate	45	-1.3268	-1.3878	-1.3192
High Acetate	60	-1.2676	-1.443	-1.347
High Acetate	75	-1.2347	-1.4722	-1.3597
High Acetate	90	-1.2626	-1.4997	-1.3746
High Acetate	105	-1.2607	-1.5334	-1.3956
High Acetate	120	-1.0935	-1.5631	-1.4187
High Acetate	135	-1.1186	-1.5805	-1.4143
High Acetate	150	-1.1498	-1.5924	-1.4303
High Acetate	165	-1.1226	-1.6025	-1.4479
High Acetate	180	-1.1046	-1.6217	-1.4539
High Acetate	195	-1.099	-1.6209	-1.4719
High Acetate	210	-1.0889	-1.6396	-1.4835
High Acetate	225	-1.1849	-1.6519	-1.5025
High Acetate	240	-1.186	-1.6548	-1.5019
High Acetate	255	-1.1826	-1.6746	-1.5132
High Acetate	270	-1.1569	-1.6301	-1.5756
High Acetate	285	-1.2645	-1.6972	-1.5783
High Acetate	300	-1.2245	-1.6856	-1.5776
High Acetate	315	-1.1844	-1.7109	-1.6007
High Acetate	330	-1.1935	-1.7212	-1.6082

Table A 16 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
High Butyrate	0	-1.1651	-1.2686	-1.2563
High Butyrate	15	-1.2	-1.2891	-1.268
High Butyrate	30	-1.2123	-1.3051	-1.3021
High Butyrate	45	-1.2274	-1.3675	-1.3378
High Butyrate	60	-1.2062	-1.4598	-1.3657
High Butyrate	75	-1.2294	-1.5087	-1.3995
High Butyrate	90	-1.2946	-1.5436	-1.4086
High Butyrate	105	-1.2434	-1.6037	-1.4316
High Butyrate	120	-1.2461	-1.6043	-1.4531
High Butyrate	135	-1.2368	-1.6199	-1.4609
High Butyrate	150	-1.2359	-1.6621	-1.4793
High Butyrate	165	-1.2366	-1.6789	-1.5085
High Butyrate	180	-1.2334	-1.665	-1.5192
High Butyrate	195	-1.2394	-1.6623	-1.5304
High Butyrate	210	-1.2274	-1.7392	-1.5379
High Butyrate	225	-1.1939	-1.7433	-1.5572
High Butyrate	240	-1.1866	-1.7321	-1.5703
High Butyrate	255	-1.1024	-1.7535	-1.5798
High Butyrate	270	-1.0816	-1.7603	-1.6015
High Butyrate	285	-1.0365	-1.748	-1.6127
High Butyrate	300	-1.067	-1.7422	-1.6155
High Butyrate	315	-1.037	-1.7561	-1.6251
High Butyrate	330	-1.014	-1.7428	-1.6508
High Propionate	0	-1.3968	-1.3005	-1.3188
High Propionate	15	-1.4227	-1.3207	-1.3804
High Propionate	30	-1.4337	-1.3605	-1.4044
High Propionate	45	-1.4157	-1.3807	-1.4264
High Propionate	60	-1.4409	-1.4048	-1.4461
High Propionate	75	-1.4286	-1.4544	-1.4607
High Propionate	90	-1.4135	-1.4756	-1.4894
High Propionate	105	-1.4258	-1.4969	-1.4912
High Propionate	120	-1.4716	-1.5307	-1.4995
High Propionate	135	-1.4588	-1.5338	-1.5094
High Propionate	150	-1.4492	-1.5542	-1.512
High Propionate	165	-1.4497	-1.5756	-1.5365
High Propionate	180	-1.5029	-1.5875	-1.5468
High Propionate	195	-1.4631	-1.5966	-1.5493
High Propionate	210	-1.4688	-1.6163	-1.5697
High Propionate	225	-1.4619	-1.6125	-1.5686
High Propionate	240	-1.4391	-1.6254	-1.5843
High Propionate	255	-1.4365	-1.6432	-1.6013
High Propionate	270	-1.432	-1.6352	-1.5921
High Propionate	285	-1.3982	-1.6488	-1.6053
High Propionate	300	-1.3952	-1.6553	-1.6212
High Propionate	315	-1.4515	-1.6511	-1.6367
High Propionate	330	-1.4361	-1.6513	-1.6171

Table A 17 Effect of treatment*time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa.

Treatment	Time	pH 1.5	pH 4.0	pH 7.0
VFA Mix	0	-1.2135	-1.1998	-1.2582
VFA Mix	15	-1.2483	-1.2345	-1.2958
VFA Mix	30	-1.2759	-1.2635	-1.33
VFA Mix	45	-1.2422	-1.316	-1.3571
VFA Mix	60	-1.2522	-1.3565	-1.3762
VFA Mix	75	-1.2588	-1.399	-1.3995
VFA Mix	90	-1.2782	-1.4315	-1.4193
VFA Mix	105	-1.2811	-1.4497	-1.4425
VFA Mix	120	-1.292	-1.4764	-1.4814
VFA Mix	135	-1.2901	-1.4938	-1.4886
VFA Mix	150	-1.3017	-1.5339	-1.4981
VFA Mix	165	-1.2855	-1.5327	-1.5189
VFA Mix	180	-1.2832	-1.5584	-1.5267
VFA Mix	195	-1.2747	-1.5602	-1.5319
VFA Mix	210	-1.2735	-1.5761	-1.5637
VFA Mix	225	-1.2632	-1.5962	-1.5722
VFA Mix	240	-1.257	-1.5936	-1.5767
VFA Mix	255	-1.2382	-1.6194	-1.6027
VFA Mix	270	-1.2328	-1.6179	-1.6011
VFA Mix	285	-1.2164	-1.6316	-1.6219
VFA Mix	300	-1.206	-1.6376	-1.6326
VFA Mix	315	-1.202	-1.6598	-1.6423
VFA Mix	330	-1.1813	-1.658	-1.6584

Table A 18 Effect of time *pH at pH 1.5, 4.0 and 7.0 on potential difference (PD) in equine nonglandular mucosa.

Time	pH 1.5	pH 4.0	pH 7.0
0	20.7381	20.7906	20.8983
15	20.7198	21.151	21.3725
30	20.8347	21.2746	21.6636
45	13.9554	18.6172	21.7154
60	13.3038	20.6163	21.6647
75	10.7972	19.7806	21.5703
90	9.1067	18.3657	21.5054
105	8.2496	17.5166	21.3785
120	7.647	17.0695	21.2979
135	7.2958	16.8939	21.2174
150	7.0594	16.8823	21.2303
165	7.0296	17.0043	21.2367
180	6.9225	17.1077	21.2412
195	6.8296	17.2768	21.2359
210	6.7412	17.4257	21.2381
225	6.691	17.5779	21.1052
240	6.5972	17.6886	21.0239
255	6.4894	17.7759	20.8781
270	6.3361	17.8361	20.7054
285	6.1412	17.889	20.4925
300	6.0221	17.8841	20.3825
315	5.8541	17.8441	20.2054
330	5.7496	17.9223	19.9861

Table A 19 Effect of time *pH at pH 1.5, 4.0 and 7.0 on short circuit current (Isc) in equine nonglandular mucosa.

Time	pH 1.5	pH 4.0	pH 7.0
0	42.7889	43.692	44.0698
15	41.4439	42.8209	43.1387
30	40.5534	41.9987	42.3676
45	27.5817	35.0942	41.4409
60	27.1117	36.6898	40.4187
75	22.1895	33.7031	40.0742
90	18.7678	30.1942	38.6764
105	16.7083	28.0609	37.7009
120	16.8289	26.6298	36.8031
135	15.8217	25.9653	36.4009
150	15.1456	25.4409	35.8476
165	14.8156	25.1987	35.2298
180	14.0173	24.9564	34.7609
195	13.4639	24.9631	34.3076
210	12.9089	24.8231	33.8387
225	12.5217	24.7476	33.2031
240	12.0062	24.7098	32.7942
255	11.0845	24.5476	32.1453
270	10.3951	24.8542	31.292
285	9.6473	24.452	30.7587
300	8.8545	24.3098	30.3987
315	7.8245	24.072	29.6964
330	7.1806	24.0764	29.1898

Table A 20 Effect of time *pH at pH 1.5, 4.0 and 7.0 on logresistance (R) in equine nonglandular mucosa.

Time	pH 1.5	pH 4.0	pH 7.0
0	8.1412	8.1581	8.131
15	8.1696	8.1939	8.1717
30	8.1921	8.2182	8.2011
45	8.1763	8.2573	8.2239
60	8.1648	8.3148	8.2488
75	8.161	8.3589	8.2555
90	8.175	8.385	8.2882
105	8.169	8.412	8.3067
120	8.1425	8.4369	8.329
135	8.1366	8.4471	8.3353
150	8.1447	8.4729	8.3516
165	8.1311	8.4871	8.3694
180	8.1323	8.4994	8.3822
195	8.1223	8.5066	8.3957
210	8.1165	8.5318	8.4092
225	8.1252	8.5423	8.4208
240	8.1159	8.5483	8.4298
255	8.0928	8.5616	8.4451
270	8.0776	8.5543	8.4627
285	8.08	8.5704	8.4739
300	8.0768	8.5764	8.4814
315	8.0655	8.5855	8.4958
330	8.06	8.5871	8.5028

Table A 21 Effect of time *pH at pH 1.5, 4.0 and 7.0 on logconductance (G) in equine nonglandular mucosa.

Time	pH 1.5	pH 4.0	pH 7.0
0	-1.2326	-1.2506	-1.2215
15	-1.2635	-1.2828	-1.2584
30	-1.2818	-1.3113	-1.293
45	-1.2678	-1.3494	-1.3173
60	-1.2576	-1.4048	-1.3415
75	-1.2493	-1.4489	-1.348
90	-1.2666	-1.4759	-1.3817
105	-1.2576	-1.5074	-1.4001
120	-1.2341	-1.5282	-1.4221
135	-1.2297	-1.5418	-1.4271
150	-1.236	-1.5638	-1.4422
165	-1.2264	-1.5802	-1.4641
180	-1.2254	-1.5915	-1.4721
195	-1.2133	-1.5964	-1.4827
210	-1.2095	-1.6246	-1.5024
225	-1.2192	-1.6318	-1.5122
240	-1.2083	-1.634	-1.5201
255	-1.1881	-1.654	-1.5357
270	-1.1694	-1.6486	-1.5531
285	-1.1723	-1.665	-1.5677
300	-1.167	-1.6666	-1.5735
315	-1.1609	-1.6793	-1.5883
330	-1.1483	-1.6844	-1.5953

Vita

Rilla Evelyn Reese was born in Gallatin, Tennessee on May 25, 1984. She graduated from Gallatin Senior High School in May 2002. In August 2002 Rilla entered Western Kentucky University, where in May 2006 she graduated with a major in Agriculture with an emphasis in Animal Science and a minor in Biology. In January of 2007 Rilla began her graduate career at the University of Tennessee with a major in Animal Science.