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PATHOGENESIS OF ACID INJURY IN THE NON-GLANDULAR REGION OF THE EQUINE STOMACH: IMPLICATIONS IN GASTRIC ULCER DISEASE

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Jenifer Ann Nadeau
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DEDICATION

This dissertation is dedicated to my parents, Mark Nadeau and Elsie Nadeau, great role models and friends, and my brothers, Bruce Nadeau and Ron Nadeau, and the rest of the family, for always believing in me, inspiring me, and encouraging me to reach higher in order to achieve my goals.

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ABSTRACT

Forty-three horses were necropsied to determine if volatile fatty acids (VFAs) produced from fermentation of carbohydrates (acetic, butyric, propionic, and valeric acids) cause cellular injury which leads to gastric ulceration when exposed to the non-glandular mucosa of the stomach at pH 1.5, 4 and 7. In part I of the study thirty horses were necropsied to determine if acetic, butyric, or propionic acid could cause cellular injury. In part 2 of the study thirteen horses were necropsied to determine if acetic, butyric, propionic or valeric acid could cause cellular injury. In both studies the stomach was removed within one hour of death and dissected along the greater curvature. A 3 cm^2 piece of non-glandular tissue was mounted mucosal side up in an Ussing's chamber. 60 mM of each VFA (acetic, butyric, propionic, or valeric acid) was combined with normal Ringer's solution and each VFA solution or normal Ringer's solution was added to the mucosal side of the chamber. Normal Ringer's solution was added to the serosal side of the chamber. The pH of the mucosal solution was changed at 30 minutes to pH 1.5, 4 or 7 and exposed for three hours. The solution on the mucosal side was drained and replaced with normal Ringer's solution at pH 7.0 at the end of three hours. Spontaneous potential difference (PD) across tissue and short-circuit current (Isc) (current necessary to nullify PD = sodium transport across tissue) were measured every 15 minutes throughout the study. Percentage of control short-circuit current (PCIsc), resistance (R), and conductance (G) were calculated. At the end of the experiment, tissues were placed in 10% formalin and stained with hematoxylin and eosin for routine histopathologic examination. In part I of the study, Isc was decreased ($P<0.01$) and resistance was increased in tissues exposed to a low pH alone, as well as those exposed to acetic acid,

butyric acid and propionic acid at an acid pH (1.5, 4.0). Tissues exposed to HCl only and acetic acid recovered to initial Isc values. Tissues exposed to butyric acid and propionic acid solutions did not recover ($P<0.01$) to initial Isc values. Tissues exposed to pH 1.5 (33%) had the greatest percent ($P<0.05$) of histopathologic change compared to pH 4.0 (28%) or pH 7.0 (25%). In part II of the study Isc was decreased ($P<0.01$) and resistance was increased ($P<0.01$) in tissues exposed to acetic acid, butyric acid and propionic acid compared to normal Ringer's solution at acid pHs (1.5,4.0). However, Isc and tissue resistance both decreased in tissues exposed to valeric acid. Also, valeric acid exposed tissues had lower ($P<0.01$) conductance values than acetic acid, butyric acid, propionic acid or normal Ringer's solution exposed tissues. Valeric acid (42%), propionic acid (31%) and butyric acid (28%) had the greatest percent ($P<0.01$) of histologic change. Normal Ringer's solution (26%) and acetic acid (18%) exposed tissues had the least percent ($P<0.01$) of histologic change. PH alone and volatile fatty acids in the presence of acid pH of the non-glandular mucosa of the stomach causes cellular swelling that may lead to necrosis and ulceration.

TABLE OF CONTENTS

CHAPTER	PAGE
PART 1: GENERAL INTRODUCTION AND OVERVIEW	
1. Review of the Literature	2
2. Rationale: Why study volatile fatty acids in acid injury?	28
3. Objectives	30
Literature Cited	32
PART II: PATHOGENESIS OF ACID INJURY IN THE NON-GLANDULAR EQUINE STOMACH: STUDIES WITH ACETIC, BUTYRIC, AND PROPIONIC ACIDS	
1. Abstract	42
2. Introduction	44
3. Materials and Methods	46
4. Results	52
5. Discussion	94
Literature Cited	99
PART III: PATHOGENESIS OF ACID INJURY IN THE NON-GLANDULAR EQUINE STOMACH: STUDIES WITH ACETIC, BUTYRIC, PROPIONIC AND VALERIC ACIDS	
6. Abstract	102
7. Introduction	104
8. Materials and Methods	106
9. Results	112
10. Discussion	161
Literature Cited	167
APPENDIX	169
VITA	182

LIST OF TABLES

TABLE		PAGE
1-1	Classification of VFAs	2
2-1	Mean concentration of volatile fatty acids found in the stomach of 29 horses	52
2-2	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa	55
2-3	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on percentage of short-circuit current in exposed equine non-glandular mucosa	62
2-4	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa	69
2-5	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa	76
2-6	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa	85
2-7	Effect of propionic Ringer's solution exposure on absorption into non-glandular mucosal tissue	90
2-8	Concentration of VFAs present after exposure to butyric Ringer's solution into non-glandular mucosal tissue	90
2-9	Effect of pH on butyric Ringer's solution exposure on absorption into non-glandular mucosal tissue	91
2-10	Effect of treatment and pH after exposure on butyric acid absorption into non-glandular mucosal tissue.	91
2-11	Effect of pH on non-glandular mucosal tissue change	93
2-12	Effect of treatment on non-glandular mucosal tissue change	93

2-13	Differences in depth of penetration of non-glandular mucosal tissue change	93
3-1	Mean concentration of volatile fatty acids found in the stomach of 29 horses	112
3-2	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa	115
3-3	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on percentage of short-circuit current in exposed equine non-glandular mucosa	123
3-4	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa	131
3-5	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa	139
3-6	Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa	150
3-7	Effect of propionic Ringer's solution exposure on absorption into non-glandular mucosal tissue	157
3-8	Concentration of VFAs present after exposure to butyric Ringer's solution into non-glandular mucosal tissue	157
3-9	Effect of pH on butyric Ringer's solution exposure on absorption into non-glandular mucosal tissue	157
3-10	Effect of treatment and pH after exposure on butyric acid absorption into non-glandular mucosal tissue.	159
3-11	Effect of pH on non-glandular mucosal tissue change	159
3-12	Effect of treatment on non-glandular mucosal tissue change	160
3-13	Differences in depth of penetration of non-glandular mucosal tissue change	160

A-1	Breakdown of horses in part I of the study by age	170
A-2	Breakdown of horses in part I of the study by breed	170
A-3	Breakdown of horses in part I of the study by sex	171
A-4	Breakdown of horses in part I of the study by reason for euthanasia	171
A-5	Breakdown of horses in part I of the study by *Gastrophilus intestinalis* larvae present in the stomach	172
A-6	Breakdown of horses in part I of the study by ulceration	172
A-7	Breakdown of horses in part II of the study by age	173
A-8	Breakdown of horses in part II of the study by breed	173
A-9	Breakdown of horses in part II of the study by sex	174
A-10	Breakdown of horses in part II of the study by reason for euthanasia	174
A-11	Breakdown of horses in part II of the study by *Gastrophilus intestinalis* larvae present in the stomach	175
A-12	Breakdown of horses in part II of the study by ulceration	175

LIST OF FIGURES

FIGURE		PAGE
Figure 2-1	Mean Short-Circuit Current - pH 1.5	54
Figure 2-2	Mean Short-Circuit Current - pH 1.5 & 7.0	54
Figure 2-3	Mean Short-Circuit Current – pH 4.0	57
Figure 2-4	Mean Short-Circuit Current - pH 4.0 & 7.0	58
Figure 2-5	Mean Short-Circuit Current - pH 7.0	58
Figure 2-6	Mean Short-Circuit Current: Acetic Ringer's Solution	59
Figure 2-7	Mean Short-Circuit Current: Butyric Ringer's Solution	59
Figure 2-8	Mean Short-Circuit Current: Normal Ringer's Solution	60
Figure 2-9	Mean Short-Circuit Current: Propionic Ringer's Solution	60
Figure 2-10	Mean Short-Circuit Current Percentage of Control - pH 1.5	61
Figure 2-11	Mean Short-Circuit Current Percentage of Control - pH 4.0	64
Figure 2-12	Mean Short-Circuit Current Percentage of Control - pH 7.0	66
Figure 2-13	Mean Short-Circuit Current Percentage of Control: Acetic Ringer's Solution	66
Figure 2-14	Mean Short-Circuit Current Percentage of Control: Butyric Ringer's Solution	67
Figure 2-15	Mean Short-Circuit Current Percentage of Control: Propionic Ringer's Solution	67
Figure 2-16	Mean Potential Difference - pH 1.5	68

Figure 2-17	Mean Potential Difference - pH 4.0	71
Figure 2-18	Mean Potential Difference - pH 7.0	72
Figure 2-19	Mean PD: Acetic Ringer's Solution	72
Figure 2-20	Mean PD: Butyric Ringer's Solution	73
Figure 2-21	Mean PD: Normal Ringer's Solution	73
Figure 2-22	Mean PD: Propionic Ringer's Solution	74
Figure 2-23	Mean Resistance - pH 1.5	74
Figure 2-24	Mean Resistance - pH 4.0	75
Figure 2-25	Mean Resistance - pH 7.0	81
Figure 2-26	Mean Resistance: Acetic Ringer's Solution	81
Figure 2-27	Mean Resistance: Butyric Ringer's Solution	82
Figure 2-28	Mean Resistance: Normal Ringer's Solution	82
Figure 2-29	Mean Resistance: Propionic Ringer's Solution	83
Figure 2-30	Mean Conductance - pH 1.5	83
Figure 2-31	Mean Conductance - pH 4.0	84
Figure 2-32	Mean Conductance - pH 7.0	87
Figure 2-33	Mean Conductance: Acetic Ringer's Solution	88
Figure 2-34	Mean Conductance: Butyric Ringer's Solution	88
Figure 2-35	Mean Conductance: Normal Ringer's Solution	89
Figure 2-36	Mean Conductance: Propionic Ringer's Solution	89
Figure 3-1	Mean Short-Circuit Current (Comparisons with VR) - pH 1.5	114
Figure 3-2	Mean Short-Circuit Current (Comparisons with VR) - pH 1.5 & 7.0	114

Figure 3-3	Mean Short-Circuit Current (Comparisons with VR) – pH 4.0	118
Figure 3-4	Mean Short-Circuit Current (Comparisons with VR) - pH 4.0 & 7.0	118
Figure 3-5	Mean Short-Circuit Current (Comparisons with VR) - pH 7.0	119
Figure 3-6	Mean Short-Circuit Current: Acetic Ringer's Solution (Comparisons with VR)	119
Figure 3-7	Mean Short-Circuit Current: Butyric Ringer's Solution (Comparisons with VR)	120
Figure 3-8	Mean Short-Circuit Current: Propionic Ringer's Solution (Comparisons with VR)	120
Figure 3-9	Mean Short-Circuit Current: Normal Ringer's Solution (Comparisons with VR)	121
Figure 3-10	Mean Short-Circuit Current: Valeric Ringer's Solution (Comparisons with VR)	121
Figure 3-11	Mean Short-Circuit Current Percentage of Control - pH 1.5 (Comparisons with VR)	122
Figure 3-12	Mean Short-Circuit Current Percentage of Control - pH 4.0 (Comparisons with VR)	126
Figure 3-13	Mean Short-Circuit Current Percentage of Control - pH 7.0 (Comparisons with VR)	126
Figure 3-14	Mean Short-Circuit Current Percentage of Control: Acetic Ringer's Solution (Comparisons with VR)	127
Figure 3-15	Mean Short-Circuit Current Percent of Control: Propionic Ringer's Solution (Comparisons with VR)	127
Figure 3-16	Mean Short-Circuit Current Percentage of Control: Butyric Ringer's Solution (Comparisons with VR)	129
Figure 3-17	Mean Short-Circuit Current Percentage of Control: Valeric Ringer's Solution (Comparisons with VR)	129

Figure 3-18	Mean PD (Comparisons with VR) - pH 1.5	130
Figure 3-19	Mean PD (Comparisons with VR) - pH 4.0	134
Figure 3-20	Mean PD (Comparisons with VR) - pH 7.0	134
Figure 3-21	Mean PD: Acetic Ringer's Solution (Comparisons with VR)	135
Figure 3-22	Mean PD: Normal Ringer's Solution (Comparisons with VR)	135
Figure 3-23	Mean PD: Butyric Ringer's Solution (Comparisons with VR)	136
Figure 3-24	Mean PD: Propionic Ringer's Solution (Comparisons with VR)	136
Figure 3-25	Mean PD: Valeric Ringer's Solution (Comparisons with VR)	137
Figure 3-26	Mean Resistance (Comparisons with VR) - pH 1.5	138
Figure 3-27	Mean Resistance (Comparisons with VR) - pH 4.0	144
Figure 3-28	Mean Resistance (Comparisons with VR) - pH 7.0	144
Figure 3-29	Mean Resistance: Acetic Ringer's Solution (Comparisons with VR)	145
Figure 3-30	Mean Resistance: Butyric Ringer's Solution (Comparisons with VR)	147
Figure 3-31	Mean Resistance: Normal Ringer's Solution (Comparisons with VR)	148
Figure 3-32	Mean Resistance: Propionic Ringer's Solution (Comparisons with VR)	148
Figure 3-33	Mean Resistance: Valeric Ringer's Solution (Comparisons with VR)	149

Figure 3-34	Mean Conductance (Comparisons with VR)- pH 1.5	149
Figure 3-35	Mean Conductance (Comparisons with VR) - pH 4.0	153
Figure 3-36	Mean Conductance (Comparisons with VR) - pH 7.0	153
Figure 3-37	Mean Conductance: Acetic Ringer's Solution (Comparisons with VR)	154
Figure 3-38	Mean Conductance: Butyric Ringer's Solution (Comparisons with VR)	154
Figure 3-39	Mean Conductance: Normal Ringer's Solution (Comparisons with VR)	155
Figure 3-40	Mean Conductance: Propionic Ringer's Solution (Comparisons with VR)	155
Figure 3-41	Mean Conductance: Valeric Ringer's Solution (Comparisons with VR)	156

LIST OF PLATES

PLATE		PAGE
A-13	Normal tissue (10x)	176
A-14	Histologic change (10x) of non-glandular mucosa by PRS at pH 1.5	176
A-15	Histologic change (10x) of non-glandular mucosa by PRS at pH 4.0	177
A-16	Histologic change (10x) of non-glandular mucosa by BRS at pH 1.5	177
A-17	Histologic change (10x) of non-glandular mucosa by BRS at pH 4.0	178
A-18	Histologic change (10x) of non-glandular mucosa by ARS at pH 1.5	178
A-19	Histologic change (10x) of non-glandular mucosa by ARS at pH 4.0	179
A-20	Histologic change (10x) of non-glandular mucosa by NRS at pH 1.5	179
A-21	Histologic change (10x) of non-glandular mucosa by NRS at pH 4.0	180
A-22	Histologic change (10x) of non-glandular mucosa by VRS at pH 1.5	180
A-23	Histologic change (10x) of non-glandular mucosa by VRS at pH 4.0	181
A-24	Histologic change (10x) of non-glandular mucosa by VRS at pH 7.0	181

NOMENCLATURE

bwt	body weight
g	grams
h	hour
kg	kilograms
L	liters
m	meter
mg	milligrams
min	minutes
ml	milliliter
mm	millimoles
mmhos/cm^2	millimhos per square centimeter (inverse of milliohms per square centimeter)
mM	millimolar
mmoles/L	millimoles per liter
mv	millivolts
ohms/cm^2	ohms per square centimeter
µg	micrograms
µm	micrometer
µmoles/cm^2	micromoles per square centimeter
µmoles/h	micromoles per hour

Abbreviations

AG	alfalfa hay/grain diet

BH	bromegrass hay diet
CAMP	cyclic adenosine monophosphate
CCK	cholecystokinin
cIsc	corrected short-circuit current
Cl^-	chloride
CO_2	carbon dioxide
CoA	coenzyme A
DNA	deoxyribonucleic acid
EGF	epidermal growth factor
G	conductance
GIT	gastrointestinal tract
GUD	gastric ulcer disease
H^+	hydrogen ions
HCl	hydrochloric acid
Isc	short-circuit current
Na^+	sodium ions
NRS	normal Ringer's solution
NSAIDs	non-steroidal anti-inflammatory drugs
P	probability level of significance of the statistical test
PCIsc	percent of control short-circuit current
pCO_2	partial pressure of carbon dioxide
PD	spontaneous potential difference
R	electrical resistance

TID	three times daily
VFAs	volatile fatty acids
VIP	vasoactive intestinal peptide

PART I

GENERAL INTRODUCTION AND OVERVIEW

CHAPTER 1

REVIEW OF THE LITERATURE

VFA Structure and Function

Volatile fatty acids (VFAs) are one to six carbon straight or branched chain compounds. Nutritionists generally study those that contain two to five carbon atoms, including acetic, propionic, butyric, isobutyric, valeric and isovaleric acids (Table 1-1-1). These VFAs are important end products of carbohydrate fermentation in animals and man.

VFAs have numerous functions, some as yet undiscovered. These compounds are an important energy source for all herbivorous animals.[2] VFAs provide 70-80% of the energy needs in ruminants.[3] Within the pony cecum, VFAs account for 30% of digestible energy intake.[4]

Table 1-1. Classification of VFAs (abbreviated).[1]

Chemical Name	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
$CH_3CHCOOH$ CH_3	isobutyric	isobutanoic	i4:0
$CH_3(CH_2)_3COOH$	valeric	pentanoic	5:0
CH_3CHCH_2COOH CH_3	isovaleric	isopentanoic	i5:0

In addition to their role in providing energy, VFAs have been implicated in acting as a respiratory fuel,[5] decreasing glucose[6] and altering cholesterol[7] metabolism, having antitumor effects,[8] increasing gene expression[9] and increasing mucosa cell proliferation.[10] They have also been implicated in increasing ileal motility,[11] increasing blood flow of the colonic mucosa[12] and stimulating colonic fluid and sodium absorption.[13-15]

Volatile Fatty Acid Production and Metabolism

All VFAs are synthesized from pyruvate. Carbohydrate fermentation by resident stomach and intestinal microbes (bacteria or protozoa) produces pyruvate. The main cell wall component of grain and hay products, cellulose is metabolized to cellobiose then to glucose, and finally, to pyruvate.[16] Pyruvate is the precursor substance to acetate, propionate, and butyrate in most cases.

Acetate can be produced from pyruvate through two different pathways. In one case, formate lyase, an enzyme produced by many bacteria and protozoa, attaches a coenzyme (CoA) group to the second carbon of the pyruvate molecule and acetyl CoA and formate are formed. Formate is then metabolized by other bacteria to form carbon dioxide (CO_2) and hydrogen gas and acetyl CoA is hydrolyzed to acetate.

An alternative mechanism for the synthesis of acetate involves the enzyme ferredoxin reductase. Ferredoxin reductase cleaves pyruvate directly to form CO_2, hydrogen and acetyl CoA. Acetyl CoA is then hydrolyzed to form acetate.

Several pyruvate independent endogenous pathways exist for the formation of acetate. First, threonine is catabolized by threonine aldolase to acetaldehyde and glycine.

Acetaldehyde is then oxidized by acetate aldehyde dehydrogenase to form acetate. Finally, biosynthesis of cysteine from serine in bacteria and plants produces acetate as a byproduct. [17]

Measurable concentrations of VFAs are found in the stomach of non-ruminants[18-20] and are at greatest concentration in the cranial portion of the stomach. Since the pKa of these VFAs is about 4.8, at a low pH, it is believed that they become dissociated and are taken up by the stomach mucosa. This is the area where the pH is moderate enough to support growth of acidophilic bacteria such as Lactobacillus.[21] Percentage of acetate to total VFA concentration varies with diet and age in animals fed conventional diets (hay, grain)[21-22] and in wild ruminants (diets of grasses or fruits)[23] but generally makes up sixty to seventy percent of all VFAs. Acetate is predominant (80% and higher) in the stomach and small intestine of simple-stomached animals such as pigs,[24-26] young ruminants such as calves,[27] and man (also simple-stomached).[28-30]

In a study of horses fed both an alfalfa hay/grain diet (AG) and a bromegrass hay diet (BH), mean acetic acid concentration ranged from 1.10–16.31 mmoles/L in horses fed AG and from 0.64-14.36 mmoles/L in horses fed BH. Mean acetic acid concentration was highest the first 7 h after feeding the AG.[31]

Propionate production has two pathways. The dicarboxylic pathway is the major pathway for propionate production. Succinate attaches to a CoA to produce succinyl CoA and is subsequently converted to propionyl CoA. CoA is cleaved and propionate is formed.

The direct reductive (acrylate or Baldwin) pathway is a minor pathway for propionate production. It occurs mainly in bacteria that utilize lactate for energy. These

bacteria are normally found in low number but increase during lactic acidosis or when feeding a high grain diet. In this pathway, pyruvate is reduced to lactate and lactate dehydrogenase then cleaves water and forms the enol, acrylyl CoA. Ferredoxin hydrogenates acrylyl CoA to propionyl CoA that in turn loses the CoA to form propionate. Percentage of propionate to total VFA concentrations, like acetate, varies with diet in animals fed conventional diets (hay, grain)[21-22] and in wild ruminants (diets of grasses or fruits)[23] but generally makes up fifteen to twenty-five percent of all VFAs. As diet become richer in concentrates (such as grain) propionate concentration increases.

In a study of horses fed AG and BH, mean propionic acid concentration ranged from 0.13–1.08 mmoles/L in horses fed AG and from 0.03-0.37 mmoles/L in horses fed BH. Mean propionic acid concentration was higher in horses fed AG versus BH.[31]

Butyrate is formed by the combination of 2 acetyl CoAs. One CoA is cleaved to form acetoacetyl CoA that in turn is reduced to B-hydroxybutyryl CoA. A water molecule is then cleaved to form butyrate. This process is dependent on energy at a higher cost than acetate synthesis. This may explain why more acetate is present in the alimentary canal than butyrate. When a high-energy diet is fed, a higher ratio of butyrate may be produced (at least theoretically) since the microbes have more energy to expend producing the butyrate molecule. Percentage of butyrate to total VFA concentrations, like acetate and propionate, varies with diet and age in animals fed conventional diets (hay, grain)[21-22] and in wild ruminants (diets of grasses or fruits)[23] but generally makes up ten to fifteen percent of all VFAs. As diet becomes richer in concentrates (such as grain) propionate concentration increases.

In a study of horses fed both AG and BH, mean butyric acid concentration ranged

from 0.04–0.79 mmoles/L in horses fed AG and from 0.02-1.58 mmoles/L in horses fed BH. Mean butyric acid concentration decreased at a more rapid rate in horses fed BH versus AG.[31]

Valeric acid is formed when water is added to pyruvic acid to form formic acid. Formic acid combines with acetic acid, propionic acid, and four hydrogens to produce valeric acid.[21] In a study of horses fed both AG and BH, mean valeric acid concentration ranged from 0.00–0.84 mmoles/L in horses fed AG and from 0.00-1.31 mmoles/L in horses fed BH.[31]

As previously mentioned, VFAs are produced in varying concentrations in the gastrointestinal tract (GIT). In a study in pigs fed various diets, concentrations of VFAs in the stomach and small intestine were low (<20 mmoles/L).[32] In stomach samples of one and three week old pigs, valeric acid was not found and in older pigs appeared irregularly.[25] In one study in horses, concentrations of VFAs in the stomach were less than 50.4 mmoles/L.[33] Another study found concentrations of VFAs in the horse stomach to be from 10-20 mmoles/L.[34] In a study looking at the effect of different diets on VFA production in horses, VFA levels were found to be highest 2 to 5 h after feeding, with VFA concentrations generally less than 20 mmoles/L.[31]

High grain diets affect VFA production. In one study, molar percent of propionate, isovalerate and valerate increased and molar percent of acetate decreased with high grain feeding.[35] In a study of horses fed both AG and BH, acetate and propionate were higher in horses fed AG versus BH.[31] There have also been a number of studies conducted which concluded that sources of dietary fiber (cellulose, oat husk, wheat bran, oat bran, pea fiber, linseed fiber, wheat fiber, etc.) influence individual VFA

concentrations.[36-37]

Absorption of VFAs

Volatile fatty acids are entirely digestible since they are monomeric in the lumen in the aqueous phase. In any area of the mammalian gastrointestinal tract, VFAs can readily be taken up by the mucosa.[38] They freely diffuse across the mucosal cytosol and enter the venous blood in the dissociated free fatty acid form.[1]

Several tissue types can absorb volatile fatty acids. The forestomach of ruminants is lined with stratified squamous epithelium.[39-42] The small intestine is lined with columnar simple epithelium. However, according to previous research, differences in epithelial structure between compartments of the forestomach,[40-42] small intestine[43-47] and the large intestine[48] in ruminants, have no effect on their respective abilities to absorb VFAs. There is little information available concerning non-ruminant gastric absorption. The cranial or oral part of the stomach of pigs, rabbits, horses and laboratory rodents is lined with a non-glandular stratified squamous epithelium similar to the forestomach of ruminants.[41-42] Therefore, some VFA absorption may occur in the stomach of non-ruminant species.[1]

An *in vitro* study using equine squamous mucosa tissue preparations revealed a 3.5 ± 3.5 μmol/cm^2 VFA loss from the lumen side of the stomach with a 0.06 ± 0.06 μmol/cm^2 gain of VFA on the blood side in a 2.5 h experimental period. This occurred when a Ringer's solution containing a 90 mM equimolar mixture of acetate, propionate, and butyrate was used to bathe the luminal tissue surface.[34] Gastric glandular mucosa revealed a 23.4 ± 9.4 μmol/cm^2 VFA loss from the lumen side of the stomach with a 0.6

$\pm$ 0.3 μmol/cm^2 gain of VFA on the blood side. The pyloric glandular mucosa revealed a 13.7 $\pm$ 3.4 μmol/cm^2 VFA loss from the lumen side of the stomach with a 1.0 $\pm$ 0.2 μmol/cm^2 gain of VFA on the blood side. These data indicated that the stratified squamous epithelium was all but impermeable to VFAs, according to the investigators. Additionally, low tissue conductance of the stratified squamous epithelium was observed in this experiment (0.3 $\pm$ 0.06 mmho/cm^2 for stratified squamous mucosa, 2.0 $\pm$ 0.3 mmho/cm^2 for gastric glandular mucosa, and 4.7 $\pm$ 0.4 mmho/cm^2 for pyloric mucosa). This suggests that different mechanisms of absorption may be occurring which may be related to the secretions (or lack thereof, in the case of the squamous mucosa) of the different mucosa types. Squamous mucosa may have decreased conductance because it does not secrete or absorb the ions necessary to transport or exchange VFAs across the mucosa.

Passive diffusion and active transport are two mechanisms of VFA absorption.[1] Passive diffusion of VFAs occurs when VFAs are transported down a steep concentration gradient across the intestinal epithelium.[18,49] Passive transport of VFAs also occurs after protonation. One mechanism involves protons (H^+) supplied from CO_2 or bicarbonate produced from microbial fermentation of VFAs in the gastrointestinal mucosa and lumen.[50,51] Evidence that supports this mechanism includes an increase in luminal pH with bicarbonate accumulation in the colon and a decrease in partial pressure of CO_2 (pCO_2) of luminal contents.[26,52-53] Additionally, in the rabbit, an increase in luminal content pH and decrease in pCO_2 of the luminal contents was observed.[54]

A coupled carrier-mediated counter transport process of H^+ with sodium ions (Na^+) is another mechanism proposed for VFA absorption. It involves exchange of

Na^+ for H^+ across the intestine of the goat, cat, pig, calf, sheep, guinea pig and the human resulting in a high level of transport of unionized VFAs.[1] This may support the hypothesis that VFA absorption is closely coupled to water and electrolyte transport.

Diet appears to have an effect on VFA absorption in addition to production. A diet that is highly fermentable appears to increase absorption. When cows were fed a high concentrate diet, absorption of fatty acids was more rapid than on an all hay diet.[55]

Quantity of VFAs absorbed seems to be dependent on surface area.[1] In ruminants, each successive compartment of the forestomach has a greater surface area to absorb VFAs. This causes a decrease in concentration when contents of the reticulum and rumen enter the omasum and abomasum so that only small amounts reach the duodenum.[56-59] The larger the surface area, the greater the absorption of VFAs.[1]

VFAs are absorbed at a rate which increases with chain length and decreases with branching., but not when the pH is neutral or basic.[55,60] In one study, the effect of VFA chain length on absorption rate was determined by perfusing the proximal and distal colon of the guinea pig.[61] In the proximal colon, a small significant increase in absorption was found when chain length increased at pH 5.58. In rumen-fistulated sheep, as pH of rumen contents decreased, VFA absorption increased in both fasting and fed sheep.[62] VFAs may be more easily absorbed in a low pH environment such as the equine stomach. In the rumen, VFA absorption is inversely related to the pH of the contents.[63-64] Branched chain VFAs such as isobutyric and isovaleric acid may have a lower absorption rate than unbranched VFAs such as acetic acid, propionic acid, butyric acid, and valeric acid.[65]

Cellular Damage Attributed to VFAs

VFAs have been implicated in causing cellular damage in a variety of animal species. In anesthetized cats, it was shown that acetic acid increased the permeability of the gastric mucosa to H^+ and Na^+. As a result, there was inward diffusion of H^+ and the outward diffusion of Na^+ across the mucosa. This may lead to a temporary reduction in intracellular pH, intracellular hypertonicity, and mucosal swelling.[66]

It was found that VFAs reduced transepithelial potential by greater than 50% and decreased epithelial electrical conductance to about 20% of its initial value.[67] It was also hypothesized that high luminal VFA concentrations should cause an overload of cellular pH and volume regulative systems. This might cause cellular swelling and cellular or epithelial damage, which may alter the mucosal barrier so that protection against back diffusion of protons is compromised. The investigators also postulated that high carbohydrate or high concentrate diets may lead to high VFA concentrations in rumen fluid since damage (ulceration) of the abomasal epithelium is found often in animals such as sheep fed concentrate-rich diets.[67]

Other investigators found that the colonic epithelium of the pig can undergo a rapidly reversible injury due to increased concentrations of VFA and low luminal pH.[68] As the luminal pH gradually decreased, epithelial blisters formed. This resulted in the sloughing of the entire surface epithelium. It was also suggested that if migrating cells fail to repair the mucosa rapidly, aggravated tissue injury could occur due to normal fermentation processes in the animal.

In a study of horses fed both AG and BH, horses fed BH tended to have more numerous and more severe gastric ulcers.[31] These horses tended to have higher

concentrations of valeric acid and butyric acid. Horses fed AG tended to have fewer and less severe gastric ulcers. These horses tended to have higher concentrations of valeric acid, butyric acid, propionic acid and a lower pH than horses without ulcers. At a low pH, VFAs, which have a pka of 4.8, are lipid soluble and able to be absorbed by the stomach mucosa. They penetrate the epithelial lining and have been shown to damage sodium transport, resulting in cell swelling, damage and necrosis. This may result in gastric ulceration.

Equine and Porcine Stomachs: Comparative Anatomy and Physiology

The relative capacity and average absolute capacity of the equine stomach are 8.5% and 17.96 L, respectively, while the porcine stomach has a 29.2% relative capacity and an average absolute capacity of only 8 L.[69] The stomach has four main functions: to begin digestion of fat and protein, mix ingested food, store food, and release the stomach contents into the duodenum in a controlled manner. Of these functions, the last two are the most important.

Three zones of the stomach are associated with mechanical function: the fundus, the corpus, and the antrum. The fundus adapts to volume and releases and stores content so that excess pressure increase does not occur. The corpus mixes saliva and gastric juice with food. The antrum pushes food past the pyloric sphincter into the duodenum. Additionally, the stomach secretes HCl and pepsinogen into the lumen, and it secretes the hormone gastrin into the blood.

The pig's stomach lining consists of a small area of stratified squamous mucosa which surrounds the gastroesophageal junction, cardiac mucosa, proper gastric mucosa,

and pyloric mucosa in the aboral portion of the stomach. The cardiac mucosa occupies a much larger region in the pig stomach than in the stomach of other animals.

The topographical anatomy of the horse stomach includes a fairly large stratified squamous mucosa, which covers a major portion of its oral extremity, a small area of cardiac mucosa, proper gastric mucosa and pyloric mucosa.[69] The dividing line that separates the glandular region of the stomach from the non-glandular region of the stomach (stratified squamous epithelial region) is called the margo plicatus.

The squamous mucosa is composed of a basal germinal layer (stratum basale), stratum spinosum, and a superficial keratinized layer (the stratum corneum. It is believed that this non-glandular region of tissue is a major site of VFA absorption. In pigs and horses, the squamous mucosa acts primarily as a feed reservoir and does not secrete bicarbonate rich fluid.[70] In contrast, sodium, bicarbonate and chloride present in the rumen may act to neutralize VFAs in ruminants, providing a protective effect similar to that of mucus.[69]

Unless a large osmotic gradient exists between the stomach lumen and plasma, the squamous mucosa of the stomach is resistant to passive diffusion of ions and water. Some agents, such as acetic acid and butyric acid, can penetrate this barrier. These agents are poorly dissociated at the pH of the stomach and are therefore lipid soluble. This high lipid solubility enables them to diffuse rapidly across the mucosal membrane. Once inside the squamous mucosal cell, H^+ is released due to the increased intracellular pH. This H^+ release results in intracellular acidification, cell damage, histamine release and hemorrhage.[68] In addition, the cell becomes susceptible to back diffusion of H^+ found in stomach contents and further acid damage. Therefore, VFAs may predispose

the gastric squamous mucosa to acid injury by decreasing cellular resistance to H^+ back diffusion.

The glandular region of the equine stomach is covered by a 200 μm thick layer of bicarbonate-rich mucus. There is a 100,000 fold concentration gradient of H^+ across the mucus layer. Luminal pH may be 1.0 to 2.0 while at the surface epithelial cells, pH is 6.5.[71] In the glandular region of the stomach are many pits with gastric glands beneath them. Columnar epithelial and mucus-secreting cells line the luminal surface and gastric pits. The gastric glands contain parietal cells that secrete HCl, chief cells that secrete pepsinogen, mast cells that release histamine, enterochromaffin cells that release histamines and endocrine peptides, G cells that release gastrin, and D cells that release somatostatin.[71] The cardiac region secretes only mucus except in the pig where it also secretes bicarbonate in exchange for chloride. It may be that the bicarbonate layer may lead to disassociation of the VFAs and render them non lipid soluble in the glandular mucosa region. The proper gastric mucosa, which merges with the cardiac mucosa, secretes pepsinogen and HCl. The glands of the pyloric region secrete mucus and pepsinogen and contain G cells and D cells that release gastrin and somatostatin, respectively, when stimulated. Somatostatin, released by the D cell, increases in response to luminal pH decrease (increased activity) and decreases with increased pH.[72]

Physiology of Gastric Acid Secretion

There are three main phases of gastric acid secretion. The cephalic phase occurs when the animal sees food, smells food, tastes food, chews or swallows. This phase is mediated entirely by the vagus nerve. When the parietal cell is stimulated by direct

cholinergic innervation (leading to an increased parietal cell response to gastrin) and cholinergic release of gastrin by the antral G cells, the response occurs. When food is present in the stomach, the gastric phase occurs. It involves both direct cholinergic stimulation of the proper gastric glands and cholinergic release of gastrin from the pyloric glands. When food enters the duodenum, and gastrin is released from the duodenal mucosa, the intestinal phase occurs and there is cholinergic stimulation of the gastric glands.[69]

Acetylcholine, gastrin and histamine can stimulate the parietal cell of the proper gastric mucosa to secrete acid (HCl). Acetylcholine may also cause the pyloric gland gastrin cells to secrete gastrin due to cholinergic control. Histamine, produced by the enterochromaffin-like cells and by the mast cells in the gastric gland, stimulates HCl secretion by the parietal cell.[69] In contrast to other animals, horses produce HCl as early as 2 days of age.[73]

Five mechanisms can inhibit gastric acid secretion. Acidification of the stomach (gastric pH decrease to 2 or less), the most important mechanism, results in suppression of gastrin by acid acting on the D cell to inhibit further release of gastrin. Acid, fat and hypertonic solutions in the intestine inhibit gastric acid secretion. Gastric inhibitory peptide is the potent inhibitor released in response to fat or glucose in the small intestine.[69] Neural inhibition of gastric acid secretion occurs when the vagus nerve synapses with fibers that inhibit gastrin release. When there is competition for gastrin receptors on the parietal cells, hormonal inhibition by secretin or cholecystokinin (CCK) or both results. In a study examining the effect of gastrin on gastric acid secretion in horses, it was found that the horse was very sensitive to histamine stimulation, and

maximal acid secretion was produced with 16 μg/kg bwt/horse compared to 64 μg/kg bwt in dogs and 4000 μg/kg bwt in rats.[74] Histamine Type-2 receptor antagonists inhibit acid secretion in response to gastrin and cholinergic action, and inhibit histamine directly as well as block its receptors. A study of the effect of nervous excitation on acid secretion in cannulated horses found that cholinergic excitation might play a role in the stimulation of volume and acid secretion in the horse.[75] It also found that insulin-induced hypoglycemia greatly inhibited basal acid output and volume secreted from stomach. This may hypothetically be attributed to inhibitory impulses carried in peptide neurons of the vagal nerves or to inhibitory impulses in adrenergic nerves acting on the parietal cells either directly or indirectly.

Gastric Ulcers in Horses

Gastric ulcers are quite prevalent in horses. Gastric lesions have been found in 25-51% of foals depending on location within the stomach, age, and any coexisting clinical disorders.[76-80] In a survey of pleasure and performance horses, 66% of 195 mature horses necropsied in Hong Kong had gastric ulcers. Eighty percent of these horses that were in active training had gastric ulcers, while a lower percentage of horses not in race training had gastric ulcers.[81] In Southern California, 81% of race horses in active training were found to have gastric ulcers and poor performance was correlated with increasing ulcer severity.[82] Sixty-seven Thoroughbred race horses, aged 2 to 9 years, were examined gastroscopically at a Maryland race track and 35 reexamined 2-3 months later. Of the 67 horses, 93% had one or more lesions present in the gastric mucosa. Lesions were present in all of the horses that raced. Of the 35 horses that were

reexamined, 91% had gastric lesions on the first examination and all had lesions on the second examination. When lesions were graded for severity on a scale of 0 to 10, the mean maximum squamous mucosal lesion score was found to be greater ($P<0.01$) for the second (4.89) than for the first examination (3.63). Also, mean maximum gastric squamous mucosal lesion score was greater ($P<0.01$) in horses that had raced (4.51) than for horses that had not raced (2.36) in the 2 months before the examination.[83] In a recent study in Standardbred racehorses in Quebec, Canada, found that 63% of Standardbred racehorses in active race training had gastric ulcers and that poor body condition and being a trotter versus a pacer significantly increased the horse's chance of gastric ulceration.[84]

Diagnosis of gastric ulcer disease (GUD) is based on clinical signs, endoscopic examination and response to treatment. Clinical signs of GUD include bruxism (grinding of teeth), ptyalism (excessive salivation), colic, poor performance, gastric reflux, depression, lack of appetite and abdominal pain (sometimes resulting in dorsal recumbency).[82,85-87] Horses displaying clinical signs of GUD are likely to have a significantly greater prevalence and severity of lesions than normal horses.[88]

Endoscopic examination is usually needed to confirm presence of gastric ulcers, determine location and severity of the lesions, and analyze treatment response. A 2 m long endoscope is needed for gastroscopy in the adult horse. However, a 110 m long endoscope may be sufficient for use in foals 30-40 days of age.[70] The endoscope is inserted down the esophagus into the stomach and the glandular and non-glandular regions of the stomach are evaluated for gastric lesions.

Gastric lesions are found primarily along the margo plicatus of the stomach in the

squamous mucosa. One investigator postulated that this may be an "anatomical locus minoris reistentiae."[89] In foals ≤ 60 days of age, lesions were generally most commonly observed in the stratified squamous epithelium adjacent to the margo plicatus rather than the glandular mucosa. Lesions sometimes occurred in the glandular mucosa in foals with other clinical disorders such as rotavirus-induced diarrhea or umbilical septicemia. In older foals, lesions were more prevalent along the margo plicatus and the lesser curvature in the stratified squamous mucosa,[79] as well as surrounding the cardia.[78] This may be due to increased maturity in mucosal protective factors in older animals.

There are several types of agents available to treat gastric ulcers in horses. These include antacids, H_2 receptor antagonists, sucralfate, and omeprazole. These treatments act by differing mechanisms.

Antacids have not yet been determined to be effective in treating gastric ulcers. They may decrease clinical signs but have not been shown to be effective in either treatment or prevention of recurrence of ulcers. Types of antacids include aluminum hydroxide, magnesium hydroxide, and calcium carbonate. Most are mixtures of aluminum hydroxide and magnesium hydroxide. Magnesium salts and calcium carbonate act rapidly to neutralize acid but have a short duration of effectiveness. In horses whose ulcers were previously healed with the use of an H_2 antagonist, 200–250 ml three times daily (TID) of magnesium/aluminum hydroxide orally may help prevent the return of clinical signs. A dose of 180 ml of a magnesium/aluminum hydroxide antacid (Maalox® Rorer Consumer) increased pH of gastric fluid to greater than 3.0 for 15–30 min in the horse.[70] Frequency and dose for treatment of gastric ulcers with antacids must be determined.

The H_2 receptor antagonists act by suppressing HCl secretion by binding to and competitively inhibiting the parietal cell histamine type-2 receptor. This receptor normally activates adenylate cyclase to produce cyclic adenosine monophosphate (cAMP) when histamine binds to it. Treatment with type 2 receptor (H_2) antagonists in 55 horses with gastric ulceration led to endoscopically confirmed resolution of lesions or improvement in 32 horses.[90] Ranitidine (Zantac® Glaxo Pharmaceuticals) and cimetidine (Tagamet® Smith Kline and French) are two drugs of this type used in the horse. These drugs have been helpful in resolving gastric lesions in foals and adult horses.[78,85] The dosage of ranitidine is usually 7 mg/kg/TID. Ranitidine is available as a syrup and in the liquid and injectable forms. Of 32 horses treated with ranitidine**,** there was significant improvement in gastric lesion scores and in 16 horses, complete healing. However, no untreated controls were used in that study.[87] Cimetidine is not as potent as ranitidine and should be given at a maximum dose of 22 mg/kg every 6–8 hours. Cimetidine is available in tablet, liquid, and injectable forms. Cimetidine can be given intravenously at 7 mg/kg every 6 h.[70] Lower doses can relieve the discomfort of ulcers, but cannot heal gastric lesions or ulcers. Therapy should continue for 14–21 days. Healing time varies depending on the individual horse.

Sucralfate (Carafate® Marion Laboratories) is a sulfated polysaccharide, which is a combination of octasulfate and aluminum hydroxide. Its mechanism of action involves adhering to ulcerated mucosa, causing increased prostaglandin E_1 synthesis and stimulation of mucus secretion. Sucralfate may best be used in addition to H_2 antagonist treatment to aggressively suppress acid secretion in addition to increasing mucosal protective factors.[70] It is available as a large tablet and is used at a dose of 20

mg/kg/TID. It also inactivates pepsin and adsorbs bile acids. However, a study was conducted comparing sulcralfate (22.0 mg/kg bwt) to corn syrup for 14 days. On day 15, it was discovered upon gastroscopic examination that sucralfate did not promote greater healing than corn syrup.[91]

Omeprazole, a substituted benzimidazole and a proton pump inhibitor, acts by blocking secretion of H^+ at the parietal cell membrane H^+/K^+ ATPase pump. This causes secretion of H^+ into the gastric lumen. The H^+/K^+ ATPase enzyme is called the proton pump of the parietal cells. Omeprazole binds irreversibly with this enzyme which may explain its long-term blockage of acid secretion. Omeprazole acts to completely suppress acid secretion and its effects can last up to 27 hours in horses depending on dose.[92] A dose of 0.5 mg/kg of omeprazole given intravenously in horses significantly decreased basal free gastric acid content and simultaneously increased pH which lasted 7 h.[93] Omeprazole (0.7 mg/kg) given once daily via nasogastric tube to 8 horses with chronic gastric cannulae was found to inhibit basal and stimulated gastric acid output by 69% and 76% respectively and increased pH from 3.2 to 4.6 in basal gastric juice by the fifth day of treatment. In the same study, stimulated gastric juice pH increased from 1.7 to 4.6 by the fifth day of treatment.[94] Seventeen Thoroughbred race horses with moderate to severe gastric ulceration were purchased within 10 days of racing and treated once daily with either nasogastric administered omeprazole (9 horses) or vehicle (8 horses) and evaluated for ulcer healing gastroscopically. In horses receiving omeprazole, gastric lesions healed significantly faster than the vehicle treated group of horses.[95] Omeprazole paste (Gastrogard, Merial Limited, NJ) is commonly given at a dosage of 4 mg/kg every 24 hours.[70]

The stomach has several protective mechanisms against damage. The mucus-bicarbonate layer covers the surface of the glandular mucosa. Prostaglandin E_2 promotes secretion of this layer and enhances mucosal blood flow.[96,97] Mucosal blood flow is important since its disruption causes ulceration.[98] Cellular restitution occurs when surface epithelial cells migrate from the gastric pit to the surface in response to an irritating substance.[71] Epidermal growth factor (EGF) is produced by the salivary glands and promotes cellular DNA synthesis and growth of gastrointestinal mucosa.[99]

The squamous mucosa has no mucus layer and responds to acid irritation by increasing the thickness of its keratin layer which is only of minimal protection from acid and pepsin.[71] Gastric lesion formation may be related to desquamation of the stratified squamous epithelium of the stomach. In a study of rats, it was found that losing these layers from the dorsal aspect of the non-glandular surface near the margo plicatus may result in susceptibility to acid injury. This loss occurred in several studies.[79,80] Replacement of these layers by the superficial cornified epithelial and keratin layers seems to be delayed in foals due to incomplete separation of the superficial layer. This could result in ulceration and erosion of the deeper layers due to continued exposure to gastric acid especially in the lesser curvature.[78]

Few exact causes of gastric ulceration have been determined, but many possible mechanisms exist. Excessive exposure to HCl may be the primary cause of squamous mucosal ulceration. According to one investigator, HCl and pepsin are the two most important contributors to gastric mucosal ulceration.[71] Pepsinogen is released from the chief cells of the gastric gland and is enzymatically cleaved at a pH of less than 3.0 to form pepsin. This formation of pepsin is known to be stimulated by secretin, and

vasoactive intestinal peptide (VIP).[100]

Glandular mucosal ulceration requires acid, but is probably due to failure or disruption of mucosal protective factors.[71] Non-steroidal anti-inflammatory drugs (NSAIDs) may be involved in the latter mechanism. NSAIDs result in ulceration of glandular and squamous mucosa in foals.[101] Also, gastric mucosal damage was found upon necropsy in 6 of 8 mature horses given large doses of phenylbutazone.[102] NSAIDs inhibit cyclooxygenase which in turn inhibits prostaglandin E_2 production, which causes increased acid secretion, decreased mucosal blood flow, and disruption of the mucus-bicarbonate barrier. However, one study of Southern California racehorses showed no correlation between use of NSAIDs and gastric ulceration. The use of furosemide and gastric ulceration did correlate, resulting in less severe gastric lesions. Furosemide is known to have effects in the pulmonary vasculature. This suggests that it may have effects on the gastric vasculature as well, increasing blood flow to the area. Disrupted blood flow may play a role in gastric ulceration.[82]

Stomach pH has also been implicated as a cause of gastric ulceration. In a study of 20 adult horses, mucosal surface pH was measured in the stomach immediately postmortem using a pH probe. The squamous epithelium had a lower pH than the glandular mucosa, with the lowest readings near the margo plicatus where most ulcers naturally occur. Fifty percent of the horses with moderate to severe ulceration had a significantly lower pH than horses with mild or no ulceration.[103] Whereas, a similar study in foals showed no difference in the mucosal surface pH in the same region of the stomach in 9 foals with lesions and 9 foals without lesions. No untreated control horses were used.[73]

Helicobacter pylori is considered to be the primary cause of peptic ulcers in people, but until recently had not been found in any equine species.[71] However, in a recent study, DNA was isolated from the glandular and squamous epithelia of seven horse stomachs and Helicobacter infection was determined using PCR to amplify the urel gene, which is specific for Helicobacters found in the stomach. Urel PCR products were amplified from both the squamous epithelium of 3 horses including 2 with squamous erosions and from one horse with glandular epithelial erosions. This is the first study that found a possible Helicobacter infection in the equid stomach, and it suggests that Helicobacter spp. may be involved in the etiology of equine gastric ulcer syndrome.[104] Many other types of bacteria have been associated with lesions in the equine, but none appear to be primarily pathogenic.

Stress has also been implicated as a cause of gastric ulcers. Stressors in laboratory animals caused ulceration of the glandular mucosa. The cause was apparently high amplitude, low velocity muscular contractions of the stomach with decreased focal mucosal capillary blood flow resulting in acid injury.[71] This mechanism may occur in foals, since 35% of 16 foals that were stressed due to concurrent clinical illness had glandular gastric ulceration.[105] It was recently postulated that elite, heavy use western performance horses were exposed to a greater intensity of stressors, since they are transported more frequently than racehorses, for longer distances, and are used more often. Many western performance horses travel from one event to the next throughout the rodeo season, basically living out of their horse trailers. However, the rate (40%) and severity of gastric ulceration in elite, heavy use western performance horses is lower than that found in horses in active race training (up to 93%).[106]

Murray and associates also found a greater prevalence and severity of lesions in horses in training. This may be due to the effect of strenuous exercise on gastric physiology or stress. Exercise could cause gastric ulceration by delaying gastric emptying and/or increasing gastric acid secretion. This may lead to increased exposure of the squamous mucosa to harmful acid, resulting in ulceration.[88]

A study of 6 cannulated mares evaluated the effect of diet on gastric ulcers when horses were fed either BH or AG. Horses fed BH tended to have more numerous and more severe gastric ulcers than horses fed AG. Horses fed AG who did get ulcers tended to have higher concentrations of butyric acid, propionic acid, valeric acid, and lower pH than horses who did not have ulcers when fed AG.[31] It was postulated that alfalfa hay may have a protective effect on the mucosa due to its calcium or protein concentration.

In pigs, diet texture has been found to be one of the major causes of gastric ulceration. A more finely ground diet resulted in a lower pH in the esophageal region and a higher pH in the pyloric region of the stomach. It was postulated that increased incidence of ulcers in the esophageal region when fine corn was fed was due to increased acidity or increased pepsin activity in the relatively unprotected esophageal region of the stomach which is lined with stratified squamous epithelium.[107] This increased incidence of ulceration may also be due to the greater availability of carbohydrates in finely ground diets which increases microbial fermentation and organic acid production.[108]

History and Use of the Ussing's Chamber System

Hans H. Ussing received a Rockefeller Fellowship at the Donner Laboratory at the University of California in 1948. While he was staying in Berkeley, he gave a

seminar on sodium transport across the frog skin. Ussing borrowed Professor E.J. Lund's book, Bioelectric Fields and Growth, from a student at the seminar who had pointed out that the author had shown the frog skin to be a redox potential. It did not change Ussing's view that active sodium transport was the main source of frog skin potential, but he did notice that Lund and Stapp had attempted to draw electric current from frog skins by reversible lead-lead chloride electrodes. This inspired his plan to "short-circuit" the skin via suitable electrodes so that the potential drop across the skin was reduced to zero. If the bathing solutions were identical, then only actively transported ions could contribute to the current passing the skin because the flux ratio for passive ions would become one. Ussing returned to Denmark shortly after formulating this idea, so it was put aside for a while.[109]

In 1950, Ussing was invited to give an introductory talk on membrane transport problems at the 18th International Physiology Congress in Copenhagen. He felt that he needed "some striking finding that would justify" his place in the program. He looked at his list of future projects and decided on the short-circuiting experiment. He had to find a coworker right away and told Dr. K. Zerahn his plan and its significance. Zerahn joined Ussing's team.[109]

Zerahn wired up the circuit, Ussing did the glass blowing, and helped by their mechanic, designed the chamber. Within a week, they had the first results. During short-circuiting, the entire current drawn from the skin was accounted for by net sodium flux (influx minus outflux). Ussing and Zerahn had shown that active sodium transport alone was responsible for the electrical asymmetry of frog skin. Within two weeks they had enough data to confirm the first findings and to warrant publication of the results.[109]

Additionally, a new system for measuring sodium transport was established.

The idea behind Ussing's system is that since the solution bathing both sides of the tissue are identical, no net transfer of passive ions takes place. The ions from active transport continue to flow one-sidedly, therefore, the current running through the short-circuit is the results of all net transport processes.[109] If dissimilar solutions are used, a junction potential is created which can be measured in a simple experiment and corrected short circuit current (cIsc) can then be calculated. Sodium transport can still be measured in this case. Ussing also attempted to explain the relationship between potential difference (PD) and sodium transport. With a given frog skin, he showed that with increasing PD, sodium influx becomes lower and outflux higher. At 110 mv, sodium influx and outflux becomes equal. Under a given set of conditions, the maximum force that can be exerted by the sodium transport mechanism represents the PD just enough to equalize the influx and outflux.[109]

In rumen epithelium and rabbit esophageal epithelium, it was found that the living layers just below the stratum corneum function in sodium transport, powered by sodium pumps, (Na^+ K^+ ATPases) in these cells.[110,111] This establishes electrical potential (PD) and current flow (short-circuit current (Isc)) across the tissue.[112] These are parameters that can be used to measure tissue resistance (permeability) and transport activity *in vitro*. Recent studies in pig stomach have confirmed the idea that current flow across equine and porcine squamous epithelia is due to sodium transport.[113] ATPases have been histochemically located in equine stratified squamous mucosa.[114] In the stratum basale, high ATPase activity decreased as superficial layers were neared, showing that there is a gradient of metabolic activity in this mucosa similar to esophagus and rumen epithelium.

The amount of sodium transport is different when the rumen epithelium is compared to the horse stomach. Increased diffusion of sodium through the layers is higher because the mucosa lining the forestomach is much thinner. This makes salt and water absorption the main functions of the rumen.[115] Horse gastric stratified squamous epithelium has higher electrical resistance (R), ranging from 2-3000 ohm/cm^2 greater than forestomach epithelium.[110,112] A 30 mmol/l HCl concentration at pH 1.5 must be present to change R and result in histological damage to pig and rabbit stratified squamous mucosa when applied as long as 3 h to the mucosa *in vitro*.[116-117]

In a study of rabbit esophageal tissue it was found that luminal H^+ can increase PD in vivo and maintain that increase.[110] This can occur when H^+ is present in the lumen due to H^+ diffusion from the lumen to the blood. It can also occur after H^+ exposure when sodium is reintroduced into the lumen due to activation of amiloride-sensitive sodium transport. In this investigation H^+ in the esophagus also decreased R and increased bidirectional Cl^- transport.[118] Also in this study, Isc was increased due to an increase in net sodium transport.

The initial response of esophageal mucosa to moderate or low H^+ concentrations is PD. However, prolonged exposure to medium concentrations (80 mm HCl) or brief exposure to higher concentrations (120 mm HCl) results in a gradual reduction in PD toward zero. A 40-50% decrease in PD was due to decreased mucosal R with no change in active transport (normal Isc and net sodium transport).[119,120] However, an 80-100% decrease in PD decreased tissue R further and inhibited net sodium transport. This was found to be due to inhibition of the Na^+K^+ATPase enzyme.

Since short chain fatty acids are weak electrolytes that are highly lipid soluble at

low luminal pH they can rapidly diffuse across tissue barriers. In the pig, it has been shown that 60 mmoles/L acetate at pH 4.5 or 20 mmoles/L acetate at pH 2.5 resulted in an instant and irreversible elimination of the Isc. Three to four hours later there was a decrease in R. When tissue was exposed to HCl at pH 1.5, R declined and returned 50% to baseline after 1 h and 25% after 3 h. Isc decreased much slower than with acidified acetate. It took 5 h to reach zero. At pH 2.5, HCl could not decrease Isc or R.[116] The abrupt elimination of Isc may be caused by undissociated acetate rapidly penetrating the outer tissue barrier acidifying the transporting cells beneath it. This disruption in volume regulation could result in cell swelling, disruption and destruction of the outer barrier layers seen histologically, accounting for the later decrease in R. On the other hand, HCl eroded the outer layers of the tissue allowing H^+ to enter the transporting cells as acidification increased, removing volume regulation and resulting in cell swelling and necrosis.[121]

Therefore Argenzio proposed a synergistic effect of VFAs and HCl on tissue damage.[112] Increased HCl concentrations would cause more VFAs to become undissociated, increasing their absorption into the tissue. Na^+ transport of the underlying layers would be immediately abolished by VFA acidification. Therefore, sodium and water entering the cells could not be remedied by sodium pumping, so cell swelling and disruption would result. As the HCl damages the outer barriers and lowers resistance, more Na^+ and water could diffuse into the underlying layers, resulting in a vicious cycle.[112]

CHAPTER 2

RATIONALE: WHY STUDY VOLATILE FATTY ACIDS IN ACID INJURY?

Gastric ulcers are quite prevalent in horses. Their presence in horses generally results in a loss of revenue and use of the horse due to decreased performance. Increased mucosal damaging factors (decreased pH and increased acidity), decreased mucosal protective factors, strenuous exercise, stress and diet have been implicated in the cause of gastric ulcers in horses. Equine diets are primarily composed of hay and/or grain and each have variable concentrations of carbohydrates. The byproducts of carbohydrate fermentation are volatile fatty acids (VFAs), which due to their high lipid solubility, may diffuse into the non-glandular squamous mucosal cells of the stomach, causing acid injury and ulceration.

In a study of six cannulated mares fed a bromegrass hay or an alfalfa hay/grain diet, horses fed bromegrass hay tended to have a greater and more severe gastric ulcers. These horses tended to have higher concentrations of valeric acid and butyric acid (VFAs). Horses fed alfalfa hay/grain tended to have fewer and less severe ulcers. These horses tended to have higher concentrations of valeric acid, butyric acid, propionic acid and a lower pH than horses without ulcers.

The proposed mechanism for VFA injury is related to their high lipid solubility. VFAs generated from fermentable carbohydrates easily penetrate the squamous mucosal cell barrier of the non-glandular portion (gastroesophageal region) of the stomach at a low pH. In their undissociated form, VFAs are weak acids, and once inside the cell cause cell acidification, inflammation and ulceration. The squamous mucosa lining the

proximal stomach in horses may be predisposed to acid injury because of its lack of mucosal protective factors (mucus and bicarbonate). This acid injury can result in gastric ulceration.

Acid injury can be determined by measuring the potential difference(PD) and short-circuit current (Isc) across tissue using the *in vitro* Ussing's chamber system. These parameters measure tissue resistance (R), (permeability) and sodium transport. A decrease in R and disruption of sodium transport results when tissue is damaged, for example by VFAs. This disruption of volume regulation may result in cell swelling, disruption and destruction of the outer barrier layers of the tissue, and possibly even necrosis of the tissue. This study was intended to (1) measure Isc and R of non-glandular squamous mucosal tissue of horse stomachs after exposure to normal Ringer's solution (control) and VFAs (60 mm acetic, butyric, propionic and valeric acids) at three different pHs (1.5, 4 and 7) using the *in vitro* Ussing's chamber system; (2) examine the tissue after exposure to VFAs and differing pHs in the Ussing's chambers under light microscopy for histopathologic changes such as cell swelling, degeneration, and necrosis; (3) extract VFAs from the homogenized squamous mucosal tissue of the stomach after exposure to VFAs and differing pHs in the Ussing's chambers and measure tissue VFA concentrations using gas chromatography to determine what VFAs are absorbed into the tissue; and (4) obtain stomach contents from the necropsied horses and measure VFA concentrations using the gas chromatography.

CHAPTER 3

OBJECTIVES

The purpose of this study was to determine if volatile fatty acids (VFAs) (acetic, butyric, propionic and valeric acids), when exposed to the squamous mucosa at three different pHs (1.5, 4 and 7), cause cellular injury which leads to gastric ulceration in the non-glandular mucosa of the equine stomach. Specific objectives for the conduct of this study included:

1. To measure short-circuit current (Isc) and potential difference (PD) and calculate percentage of short circuit current (PCIsc), resistance (R) and conductance (G) of non-glandular squamous mucosal tissue of horse stomachs after exposure for 3 h to a normal Ringer's solution (control) and VFAs (60 mm acetic, butyric, propionic, and valeric acids) at three different pHs (1.5, 4 and 7) on the mucosal side of the tissue using the *in vitro* Ussing's chamber system.
2. To measure degree of recovery in the tissue by replacing the VFAs on the mucosal side of the tissue with normal Ringer's solution and measuring Isc and R for one hour.
3. To collect gastric juice from the stomachs of the necropsied horses immediately following necropsy and measure VFA concentrations (acetic, butyric, propionic valeric, isobutyric and isovaleric acids) using gas chromatography (collected at time of necropsy, frozen and analyzed later as a batch).
4. To examine the tissue after exposure to VFAs and the control solution under light microscopy for histopathologic changes such as cell swelling, degeneration and

necrosis. The tissue will be removed from the Ussing's chamber and immediately fixed in 10% formalin for histopathologic examination.

5. To extract VFAs from the homogenized squamous mucosal tissue of the stomach after exposure to VFAs and control solution at differing pHs in the Ussing's chamber and measure tissue VFA concentrations using gas chromatography to determine tissue VFA absorption.

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PART II

PATHOGENESIS OF ACID INJURY IN THE NON-GLANDULAR EQUINE STOMACH: STUDIES WITH ACETIC, BUTYRIC, AND PROPIONIC ACIDS

CHAPTER 1

ABSTRACT

Thirty horses were necropsied to determine if volatile fatty acids (VFAs) produced from fermentation of carbohydrates (acetic, butyric, propionic, and valeric acids) cause cellular injury which leads to gastric ulceration when exposed to the non-glandular mucosa of the stomach at pH 1.5, 4 and 7. The stomach was removed within one hour of death and dissected along the greater curvature. A 3 cm^2 piece of non-glandular tissue was mounted mucosal side up in an Ussing's chamber. 60 mM of each VFA (acetic, butyric, or propionic acid) was combined in normal Ringer's solution (NRS) and each VFA solution or NRS alone was added to the mucosal side of the chamber. Normal Ringer's solution was added to the serosal side of the chamber. The pH of the mucosal solution was changed at 30 minutes to pH 1.5, 4 or 7 and exposed for three hours. The solution on the mucosal side was drained and replaced with NRS at pH 7.0 at the end of three hours to determine tissue recovery. Spontaneous potential difference (PD) across tissue and short-circuit current (Isc) (current necessary to nullify PD = sodium transport across tissue) were measured every 15 minutes throughout the study. Percentage of control short-circuit current (PCIsc), resistance (R), and conductance (G) were calculated. Following Ussing's chamber exposure, tissues were placed in 10% formalin and stained with hematoxylin and eosin for routine histopathologic examination. Isc decreased immediately ($P<0.01$) and resistance decreased ($P<0.01$) after 150 min in tissues exposed to NRS at pH 1.5 compared to tissues exposed to NRS at pH 4.0 and 7.0. Isc decreased ($P<0.01$) and resistance increased in tissues exposed to acetic acid, butyric

acid and propionic acid at an acid pH when compared to NRS at all pHs. Tissues exposed to these solutions did not recover ($P<0.01$) to initial Isc values. Tissues exposed to pH 1.5 (33%) had the greatest percent ($P<0.05$) of histopathologic change compared to pH 4.0 (28%) or pH 7.0 (25%). In part II of the study Isc was decreased ($P<0.01$) and resistance was increased ($P<0.01$) in tissues exposed to acetic acid, butyric acid, propionic acid and valeric acid compared to normal Ringer's solution at acid pHs (1.5,4.0). HCl alone (an acid pH) and VFAs cause functional and histopathologic changes in non-glandular mucosa of the horse stomach. These tissue changes appear to be irreversible. Volatile fatty acids cause cell swelling that may lead to necrosis and ulceration when exposed to the non-glandular mucosa at acid pHs (all but acetic acid), and neutral pH (valeric acid only).

CHAPTER 2

INTRODUCTION

Equine gastric ulcer syndrome (EGUS) is common in horses and foals. The exact pathogenesis is unknown. However, volatile fatty acids and pH have been implicated as a possible cause of EGUS. In the pig, acetic acid was found to induce reversible acid injury to the non-glandular mucosa.[1] This was determined by measuring the potential difference and short-circuit current across tissue using the *in vitro* Ussing's chamber system. These parameters measure tissue resistance (tissue permeability) and sodium transport. A decrease in resistance and disruption of sodium transport resulted when tissue was damaged by acetic acid. The proposed mechanism for volatile fatty acid injury is related to their high lipid solubility. At a low pH, acetic acid, butyric acid, or propionic acid can become undissociated and easily penetrate the squamous mucosal cell barrier of the stomach. The squamous mucosa lining the proximal stomach in horses may be predisposed to volatile fatty acid injury because of its lack of protective factors (mucus and bicarbonate). Once inside the cell, these weak acids can cause cell acidification, inflammation, and ultimately, ulceration.

VFAs have been measured in the stomach of horses fed different diets. Horses fed an alfalfa hay/grain diet had a higher mean acetic acid concentration (1.10 - 16.31 mmoles/L) than a bromegrass hay diet (0.64 – 14.31 mmoles/L) in stomach contents.[2] In a study of twenty horses, acetic acid concentration ranged from 10.7 – 50.4 mmoles/L when hay was fed.[3] In the same horses, acetic acid concentration ranged from 8.4 – 25.6 mmoles/L when hay and grain was fed. Horses fed a bromegrass hay diet had a higher

mean butyric acid concentration (1.58 mmoles/L) than an alfalfa hay/grain diet (0.79 mmoles/L) immediately postfeeding in stomach contents.[2] In this same study, horses fed an alfalfa hay/grain diet had a higher mean butryic acid concentration (0.04 – 0.79 mmoles/L) than a bromegrass hay diet (0.02 – 1.58 mmoles/L) in stomach contents. In a study of twenty horses, butyric acid concentration in the stomach ranged from 0.3 - 0.9 mmoles/L when hay was fed.[3] In the same horses, butyric acid concentration in the stomach ranged from 0.6 – 1.4 mmoles/L when hay and grain was fed. Horses fed an alfalfa hay/grain diet had higher mean propionic acid concentrations than a bromegrass hay diet for six hours after feeding in stomach contents.[2] In this same study, horses fed an alfalfa hay/grain had a higher mean propionic acid concentration (0.13 to 1.08 mmoles/L) than a bromegrass hay diet (0.03 to 0.37 mmoles/L) in stomach contents. In a study of twenty horses, propionic acid concentration ranged from 1.6 to 6.1 mmoles/L when hay was fed.[3] In the same horses, propionic acid concentrations in the stomach ranged from 0.5 to 1.8 mmoles/L when hay and grain was fed.

PH of stomach contents and VFAs have been implicated in causing gastric ulcers in horses. In the study of horses fed an alfalfa hay/grain diet or a bromegrass hay diet, a stepwise model showed that horses with gastric ulcers had lower stomach pH and higher concentrations of VFAs (butyric, propionic and valeric acids) than horses that did not have gastric ulcers. Thus, VFAs and pH may be important factors in the cause of gastric ulcers in horses. The purpose of the study was to determine effect of pH and VFAs (acetic, butyric, and propionic acid) on sodium transport and tissue resistance across the squamous mucosa of the horse stomach in order to elucidate their role in the pathogenesis of EGUS in horses.

CHAPTER 3

MATERIALS AND METHODS

Animals and Treatments

Thirty horses going to necropsy for reasons of debilitation and/or donation were used in this study. Information regarding sex, breed and age were obtained. The horses were humanely euthanatized by lethal injection using an overdose of barbiturate (Beuthanasia®, Schering- Plough, Kenilworth, NJ). Within 1 hour of death, the stomachs from the horses were removed and dissected along the greater curvature to expose the non-glandular mucosa. The stomach contents were removed immediately and placed in two 50 ml vials and frozen at –70° C for later analysis of VFA concentrations. The whole stomach, including non-glandular mucosa**,** was cleaned of food material using deionized water and NRS and examined for gastric ulceration and given a grade based on an equine gastric ulcer scoring system.[4] The non-glandular mucosa was then dissected free from the underlying muscular and serosal tissue and cut into approximately 3.5 cm^2 discs. The tissue was placed in an Ussing's chamber and exposed to different VFAs. After VFA exposure, tissue was removed from the chamber and divided in half. One half was placed in 10% formalin for histopathologic examination and the other half was placed in NRS and frozen at –70 C for evaluation of tissue VFA concentration.

Ussing's Chamber Techniques

Gastric tissues were studied in Ussing's chambers based on a previous study by Argenzio and Eisemann.[1] Non-glandular mucosal tissue was pinned mucosal side down in a paraffin tray and bathed in oxygenated NRS maintained at 20 to 22° C (room temperature). The non-glandular mucosa was separated from the underlying muscle layers mounted in Ussing's chambers with an aperture of 7.07 cm^2**.** Tissues were bathed in 10 ml of Normal Ringer's Solution (NRS) on the mucosal and serosal surfaces and were oxygenated by circulating the solutions in water-jacket reservoirs with a gas lift bubbled with 95%O_2-5% CO_2. The spontaneous potential difference (PD) was measured by use of Ringer's agar bridges, whose composition was identical to the serosal solution bathing the tissue. The PD was nullified by use of an automatic voltage clamp through Ag-AgCl electrodes. Tissue resistance was calculated from the open circuit PD and from the current necessary to nullify the PD, the short-circuit current (Isc). Calomel electrodes filled with and bathed in saturated KCl were used to record the PD and Isc.

For the study, control tissues were bathed in 10 ml of NRS, with the following composition: in mM: 142 Na, 124 Cl, 25 HCO_3, 10 glucose, 5 K, 1.65 HPO4, 1.25 Ca, 1.1 Mg, and 0.3 H2PO4, on the mucosal and 10 ml of NRS on the serosal surface. For the solutions containing acetic, butyric, or propionic acids, the sodium salt of the VFA was substituted for an equivalent of sodium chloride so that the final concentration was 60 mM in NRS resulting in an acetic Ringer's solution, butyric Ringer's solution and propionic Ringer's solution. The solution containing the VFA (10 ml) was added to the mucosal side of the tissue and 10 ml of NRS was added to the serosal side of the tissue. Since dissimilar solutions were used in the mucosal and serosal baths, liquid junction

potentials (caused by net diffusion of sodium ions from solution to bridge to electrode resulting in PD changes) were present during the electrical measurements. To determine their magnitude and orientation, external circuits were created using the two experimental solutions as described by Orlando et al.[5] Briefly, a KCl bridge connected the chambers. A Ringer's agar bridge connected the calomel electrode to the first solution (NRS). A Ringer's agar bridge also connected the other calomel electrode to the VFA solution. First, the second solution was NRS and the voltage was recorded as the zero voltage. Next, the second solution was changed to the VFA solution and the agar bridge was replaced. Voltage was recorded and this was subtracted from the zero voltage. The result was the junction potential between the two solutions.

The pH (7.0, 4.0, 1.5) of the bathing solution was adjusted by titration with 1 N HCl while measuring with a pH electrode (Accumet A-10 portable pH meter, Fisher Scientific, Pittsburgh, Pa.). For each VFA, chambers were set up with a pH of 1.5, 4, or 7 on the mucosal side for a total of 3 chambers per VFA per experiment. Tissue PD and Isc were measured when each of the three VFAs was studied. Measurements were taken every 15 minutes, and each study lasted for 3.5 hours. The pH of the solution was checked hourly to ensure stability.

At the end of the experiment, conductance, resistance, corrected Isc (corrected for presence of junction potentials), and percent of control Isc were calculated. All of the calculations were based on Ohm's law, V(voltage)=I(current(amps)) x R(resistance(ohms)).

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

Resistance (R): inverse of conductance x 1000

Short-circuit current (Isc) : Isc/ resistance x 1000

Percentage of control Isc: ((cIsc VFA Ringer's solution)/(cIsc normal Ringer's solution)) x 100

VFA Analysis of Tissue and Gastric Contents

To determine uptake of VFAs into the non-glandular mucosal tissues, the previously frozen one-half disc of tissue was thawed and analyzed by gas chromatography using the following procedure. The tissue was weighed, diced (cut into tiny pieces with a razor) and placed in 5 ml of deionized water. Samples were then homogenized for 5 minutes using the Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Samples were transferred into 15 ml Beckman polypropylene centrifuge tubes and centrifuged at 13,000 rpm for 20 minutes. A 2 ml aliquot was removed from the supernatant and placed into a 5 ml polypropylene test tube. A 1 ml aliquot was removed from the supernatant and placed in a 1.5 ml microcentrifuge tube and cooled on ice. A 0.2 ml internal standard solution (25% phosphoric acid) was added to each aliquot, vortexed at 13,000 rpm for 20 minutes, the supernatant was removed and placed into a gas chromatography vial (~1 ml) and clamped with an aluminum vial closure. The vial was frozen at –70° C for later analysis. Concentrations of VFAs (acetic, butyric, isobutyric, propionic acid, valeric, and isovaleric) were measured in the homogenized tissue using a gas chromatography method by Playne,[6] modified by Mathew et al.[7]

Next, the previously frozen gastric contents of 29 of 43 horses (combination of 30 horses from this study and 13 horses from the next study) were thawed, and

approximately 4 g of the gastric contents were centrifuged at 15,000 x g at 4° C for 15 min in a Beckman Model J-21C Centrifuge with a JA-20 rotor (Beckman Instruments, Palo Alto, CA). One and one-half milliliters of supernatant were added to 300 µl of 25% metaphosphoric acid made from ortho-phosphoric acid (Fisher Scientific Company, Pittsburgh, PA) at a 5:1 ratio and incubated at room temperature for 30 min. After centrifugation to remove the precipitate, 1 µl of sample (either of the homogenized tissue preparation described above or the gastric contents) was injected into a Hewlett Packard Model 5890 Gas Chromatograph (Hewlett Packard, Avondale, PA) with an HP-FFAP 10 m x 0.53 mm x 1 µm capillary column packed with cross-linked polyethylene glycol-TPA. A flame ionization detector (Hewlett Packard, Avondale, PA) was used with an injector temperature of 200° C, an oven temperature of 80° C, and a detector temperature of 250° C. The carrier gas was helium with a flow rate of 30 ml/min. The linear velocity was 26 cm/sec. Head pressure was 1 psi. The standards used were acetic acid, butyric acid, isobutyric acid, propionic acid, valeric acid and isovaleric acid (Alltech Associates, Inc. Deerfield, IL) and an internal standard of 2-ethylbutyric acid (Eastman Kodak Inc., Rochester, NY). The retention times were approximately 8.38 min for acetic acid, 12.68 min for butyric acid, 11.49 min for isobutyric acid, 10.88 min for propionic acid, 15.54 min for valeric acid, 13.60 min for isovaleric acid, and 16.19 min for 2-ethylbutyric acid.

Preparation for Histopathologic Examination

The other half of the tissues placed in 10% formalin, was then prepared for histologic examination. Tissues were trimmed and embedded in paraffin, sectioned at 5 µm using a microtome, placed on glass slides, and stained with hematoxylin and eosin for

examination under light microscopy. Slides were examined under light microscopy to determine amount of cell swelling or necrosis present. Measurements were taken using the Bio Quant (R & M Biometrics Inc., Nashville, TN) computer program (True Color Windows Advanced Image Analysis System). A thickness measurement was taken from the luminal surface of the keratinized layer (stratum corneum) to the beginning of the lamina propria to determine depth of tissue (L1). Five measurements were taken along the same region of uniform tissue and a mean was calculated for each of the 2 measurements. To calculate percent cell swelling, a thickness measurement was taken across the layer of cell swelling (L2) when present. Percent cell swelling was calculated using the formula L2/L1 x 100. The location of cell swelling (stratum corneum, stratum spinosum, or stratum basale or a combination) was also recorded.

Statistical Analyses

Data were analyzed using the mixed procedure of SAS. The model used for each tissue type and variable was a randomized block design split-plot ANOVA with treatment (solution conditions) in the main plot and time and time X treatment interaction in the subplot, blocked on horse. Time was a repeated measures factor. Least squares means and pooled standard errors of the mean were calculated and a least significant difference mean separation was performed. $P < 0.05$ was considered significant.

CHAPTER 4

RESULTS

Tissues obtained from the horses used in the Ussing's chamber system were grossly normal. Information regarding age (Table A-1), breed (Table A-2), sex (Table A-3), reason for euthanasia (Table A-4), *Gastrophilus intestinalis* larvae (bots) present in the stomach (Table A-5) and ulceration present (Table A-6) was obtained. None of these characteristics was found to be a statistically significant influence on the parameters studied.

In the gastric contents collected from each stomach within 1 hour acetic acid was highest in concentration followed by isobutyric, propionic, butyric, isovaleric and valeric acids, respectively (Table 2-1).

Table 2-1. Mean concentration of volatile fatty acids found in the stomach of 29 horses.

Volatile fatty acid concentration	Mean	Standard Error
Acetic	16.23	2.77
Propionic	0.67	0.28
Butyric	0.30	0.08
Isobutyric	1.09	0.94
Valeric	0.02	0.08
Isovaleric	0.09	0.17

Acetic acid, butyric acid and propionic acid decreased ($P<0.01$) Isc following the 30 minute acclimation period at pH 1.5 (Figure 2-1 and 2-2, Table 2-2). In each case, there was some, but not complete recovery following 165 min of exposure. Isc was decreased the greatest with butyric acid exposure followed by propionic acid and acetic acid. Isc was also decreased ($P<0.01$) in normal Ringer's exposed tissues at pH 1.5, and they did not completely recover following 195 min of exposure.

Acetic acid, butyric acid and propionic acid decreased ($P<0.01$) Isc following the 30 minute acclimation period at pH 4.0 (Figure 2-3 and 2-4, Table 2-2). In each case, there was some, but not complete recovery following 195 min of exposure. Isc was decreased the greatest with butyric acid exposure, followed by propionic acid and acetic acid.

There were no significant ($P<0.05$) differences in Isc values at pH 7.0 (Table 2-2, Figure 2-5).

Acetic acid, butyric acid and propionic acid decreased ($P<0.01$) Isc at pH 1.5 and 4.0 compared to pH 7.0 (Figure 2-6, 2-7, 2-8). Normal Ringer's solution decreased ($P<0.01$) Isc at pH 1.5, followed by pH 4.0, compared to pH 7.0 (Figure 2-9).

Butyric acid decreased ($P<0.01$) PCIsc following the 30 minute acclimation period at pH 1.5 (Figure 2-10). There was some, but not complete, recovery in butyric acid exposed tissues (Table 2-3, Figure 2-10).

Butyric acid decreased ($P<0.01$) PCIsc following the 30 min acclimation period at pH 4.0 (Table 2-3, Figure 2-11). PCIsc was decreased the greatest with butyric acid exposure followed by propionic acid and acetic acid. There was complete recovery in acetic acid exposed tissues, but not in butyric acid or propionic acid.

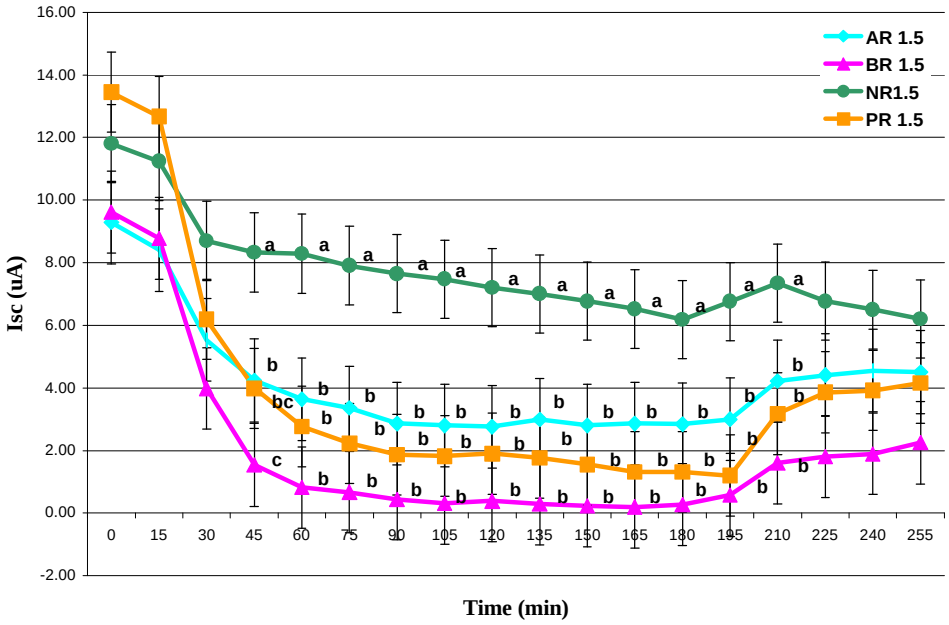


Figure 2-1. Mean Short-Circuit Current - pH 1.5

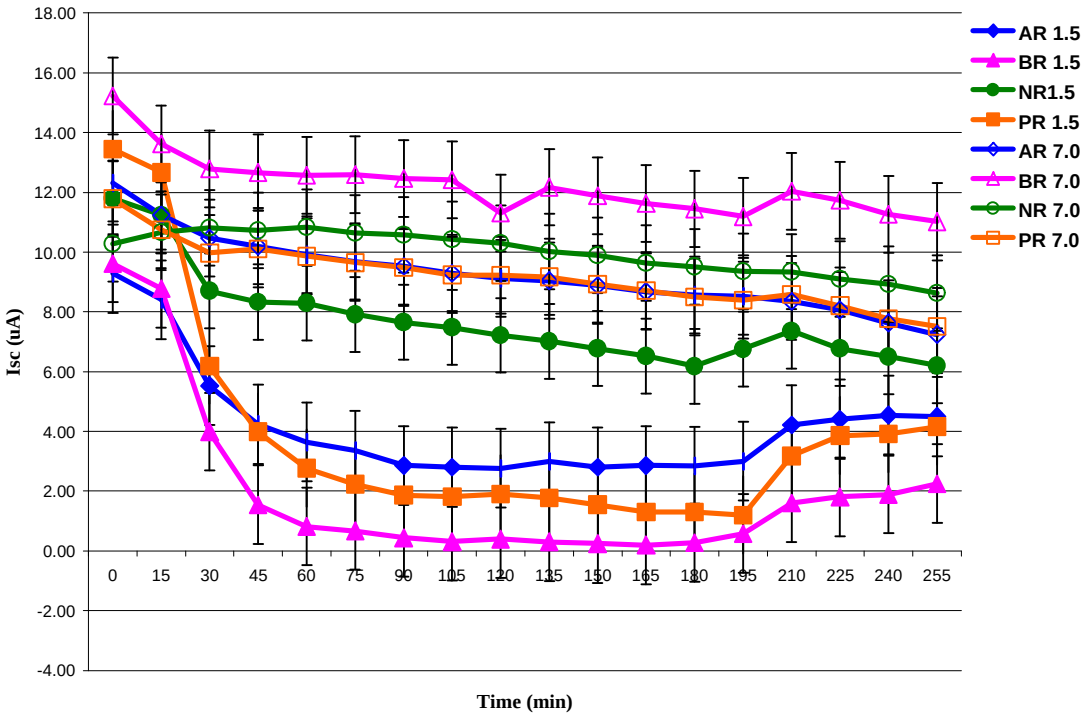


Figure 2-2. Mean Short-Circuit Current: pH 1.5 & 7.0

Table 2-2. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (μA) ±SEM @ pH 1.5	Mean (μA) ±SEM @ pH 4.0	Mean (μA) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	9.29 ± 1.32^{g}	11.79 ± 1.32^{g}	12.31 ± 1.28^{abg}
0	Butyric (n=26,26,27)	9.62 ± 1.30^{dg}	11.39 ± 1.30^{dg}	15.22 ± 1.28^{aeg}
0	Normal (n=29,26,28)	11.80 ± 1.25^{g}	9.79 ± 1.29^{g}	10.28 ± 1.27^{bg}
0	Propionic (n=28,29,27)	13.45 ± 1.28^{dg}	9.40 ± 1.26^{deg}	11.78 ± 1.28^{beg}
15	Acetic (n=26,25,28)	8.41 ± 1.32	10.96 ± 1.32	11.23 ± 1.28
15	Butyric (n=27,27,27)	8.78 ± 1.30^{d}	11.21 ± 1.30^{de}	13.62 ± 1.28^{e}
15	Normal (n=27,24,27)	11.24 ± 1.26	10.02 ± 1.30	10.66 ± 1.27
15	Propionic (n=28,29,28)	12.67 ± 1.28^{d}	8.94 ± 1.26^{e}	10.75 ± 1.28^{de}
30	Acetic (n=26,25,28)	5.53 ± 1.32^{d}	6.59 ± 1.32^{abd}	10.47 ± 1.28^{e}
30	Butyric (n=26,27,27)	3.99 ± 1.30^{d}	4.92 ± 1.30^{b}	12.78 ± 1.28^{e}
30	Normal (n=28,27,27)	8.70 ± 1.26	8.61 ± 1.28^{a}	10.81 ± 1.27
30	Propionic (n=27,29,27)	6.19 ± 1.28^{d}	5.79 ± 1.26^{abd}	9.97 ± 1.28^{e}
45	Acetic (n=25,25,27)	9.51 ± 1.35^{ad}	6.31 ± 1.32^{be}	10.20 ± 1.28^{d}
45	Butyric (n=23,26,27)	1.54 ± 1.32^{bd}	2.66 ± 1.30^{cd}	12.66 ± 1.28^{e}
45	Normal (n=27,28,28)	8.33 ± 1.26^{a}	8.93 ± 1.28^{a}	10.72 ± 1.27
45	Propionic (n=27,29,28)	3.99 ± 1.28^{bcd}	4.75 ± 1.26^{bcd}	10.10 ± 1.28^{e}
60	Acetic (n=23,26,28)	3.36 ± 1.33^{bd}	5.89 ± 1.32^{bd}	9.91 ± 1.28^{e}
60	Butyric (n=26,25,28)	0.82 ± 1.30^{bd}	1.72 ± 1.31^{cd}	12.57 ± 1.28^{e}
60	Normal (n=27,27,29)	8.29 ± 1.26^{ad}	9.07 ± 1.28^{ade}	13.60 ± 1.28^{e}
60	Propionic (n=26,29,28)	2.77 ± 1.29^{bd}	3.86 ± 1.26^{bcd}	9.86 ± 1.28^{e}
75	Acetic (n=23,26,28)	3.36 ± 1.33^{bd}	5.39 ± 1.32^{bd}	9.68 ± 1.28^{e}
75	Butyric (n=26,25,28)	0.67 ± 1.30^{bd}	1.40 ± 1.31^{cd}	12.59 ± 1.28^{e}
75	Normal (n=30,28,29)	7.91 ± 1.25^{a}	8.88 ± 1.28^{a}	10.64 ± 1.26
75	Propionic (n=27,29,28)	2.23 ± 1.28^{bd}	3.33 ± 1.26^{bcd}	9.65 ± 1.28^{e}
90	Acetic (n=26,26,28)	2.86 ± 1.32^{bd}	5.06 ± 1.32^{bd}	9.53 ± 1.28^{e}
90	Butyric (n=26,22,28)	0.44 ± 1.30^{bd}	0.92 ± 1.32^{cd}	12.46 ± 1.28^{e}
90	Normal (n=30,28,29)	7.65 ± 1.25^{ad}	8.80 ± 1.28^{ade}	10.58 ± 1.26^{e}
90	Propionic (n=26,28,28)	1.87 ± 1.29^{bd}	2.83 ± 1.27^{bcd}	9.48 ± 1.28^{e}
105	Acetic (n=26,26,28)	2.80 ± 1.32^{bd}	4.87 ± 1.32^{bd}	9.30 ± 1.28^{be}
105	Butyric (n=26,21,28)	0.31 ± 1.30^{bd}	0.92 ± 1.33^{cd}	12.42 ± 1.28^{be}
105	Normal (n=30,28,29)	7.48 ± 1.25^{ad}	8.48 ± 1.28^{ade}	10.43 ± 1.26^{abe}
105	Propionic (n=25,26,27)	1.82 ± 1.29^{bd}	2.57 ± 1.28^{bcd}	9.24 ± 1.28^{ae}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-2 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (μA) ±SEM @ pH 1.5	Mean (μA) ±SEM @ pH 4.0	Mean (μA) ±SEM @ pH 7.0
120	Acetic (n=24,26,28)	2.76 ± 1.33^{bd}	4.74 ± 1.32^{bd}	9.12 ± 1.28^{e}
120	Butyric (n=26,19,27)	0.39 ± 1.30^{bd}	0.99 ± 1.35^{cd}	11.31 ± 1.28^{e}
120	Normal (n=30,28,29)	7.21 ± 1.25^{ad}	8.04 ± 1.28^{ade}	10.30 ± 1.26^{e}
120	Propionic (n=23,26,27)	1.90 ± 1.30^{bd}	2.38 ± 1.28^{bcd}	9.22 ± 1.28^{e}
135	Acetic (n=25,26,28)	2.99 ± 1.32^{bd}	4.65 ± 1.32^{bd}	9.04 ± 1.28^{be}
135	Butyric (n=25,18,27)	0.30 ± 1.31^{bd}	0.92 ± 1.35^{cd}	12.17 ± 1.28^{be}
135	Normal (n=30,28,29)	7.01 ± 1.25^{ad}	7.87 ± 1.28^{ade}	10.02 ± 1.26^{abe}
135	Propionic (n=23,27,27)	1.77 ± 1.30^{bd}	2.20 ± 1.27^{bcd}	9.17 ± 1.28^{e}
150	Acetic (n=25,26,28)	2.80 ± 1.32^{bd}	4.59 ± 1.32^{bd}	8.89 ± 1.28^{e}
150	Butyric (n=24,20,27)	0.24 ± 1.31^{bd}	0.70 ± 1.34^{cd}	11.88 ± 1.28^{e}
150	Normal (n=30,28,29)	6.77 ± 1.25^{ad}	7.76 ± 1.28^{ae}	9.89 ± 1.26^{d}
150	Propionic (n=26,26,27)	1.55 ± 1.29^{bd}	2.14 ± 1.28^{bcd}	8.93 ± 1.28^{e}
165	Acetic (n=26,26,28)	2.86 ± 1.32^{bd}	4.52 ± 1.32^{bd}	8.68 ± 1.28^{e}
165	Butyric (n=23,19,27)	0.20 ± 1.32^{bd}	0.71 ± 1.35^{cd}	11.63 ± 1.28^{e}
165	Normal (n=30,28,29)	6.52 ± 1.25^{ad}	7.31 ± 1.28^{ade}	9.64 ± 1.26^{e}
165	Propionic (n=25,27,27)	1.31 ± 1.29^{bd}	1.88 ± 1.27^{bcd}	8.71 ± 1.28^{e}
180	Acetic (n=25,26,28)	2.84 ± 1.32^{bd}	4.52 ± 1.32^{bd}	8.56 ± 1.28^{e}
180	Butyric (n=25,19,27)	0.27 ± 1.31^{bd}	0.69 ± 1.35^{cd}	11.45 ± 1.28^{e}
180	Normal (n=30,28,29)	6.18 ± 1.25^{ad}	7.29 ± 1.28^{ade}	9.51 ± 1.26^{e}
180	Propionic (n=25,27,27)	1.31 ± 1.29^{bd}	1.96 ± 1.27^{bcd}	8.50 ± 1.28^{e}
195	Acetic (n=25,24,26)	3.00 ± 1.32^{bd}	4.70 ± 1.33^{bd}	8.52 ± 1.29^{e}
195	Butyric (n=22,18,24)	0.58 ± 1.33^{bd}	0.96 ± 1.35^{cd}	11.19 ± 1.30^{e}
195	Normal (n=29,27,28)	6.76 ± 1.25^{a}	7.02 ± 1.28^{a}	9.36 ± 1.27
195	Propionic (n=24,26,26)	1.20 ± 1.30^{bd}	1.81 ± 1.28^{bcd}	8.39 ± 1.29^{e}
210	Acetic (n=26,26,28)	4.22 ± 1.32^{bd}	4.18 ± 1.32^{bd}	8.35 ± 1.28^{be}
210	Butyric (n=25,20,27)	1.60 ± 1.31^{bd}	1.23 ± 1.34^{cd}	12.03 ± 1.28^{ae}
210	Normal (n=29,28,29)	7.35 ± 1.25^{a}	6.44 ± 1.28^{a}	9.34 ± 1.26^{ab}
210	Propionic (n=24,25,26)	3.18 ± 1.30^{bd}	2.09 ± 1.28^{bcd}	8.59 ± 1.29^{be}
225	Acetic (n=26,26,28)	4.41 ± 1.32^{d}	4.68 ± 1.32^{bd}	8.06 ± 1.28^{be}
225	Butyric (n=25,25,28)	1.81 ± 1.31^{d}	1.59 ± 1.31^{cd}	11.74 ± 1.28^{ae}
225	Normal (n=30,28,29)	6.77 ± 1.25	6.45 ± 1.28^{a}	9.10 ± 1.26^{ab}
225	Propionic (n=26,25,27)	3.86 ± 1.29^{d}	2.72 ± 1.28^{bcd}	8.21 ± 1.28^{be}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-2. (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (μA) ±SEM @ pH 1.5	Mean (μA) ±SEM @ pH 4.0	Mean (μA) ±SEM @ pH 7.0
240	Acetic (n=25,26,28)	4.55 ± 1.32^{d}	5.19 ± 1.32^{bde}	7.63 ± 1.28^{be}
240	Butyric (n=27,28,28)	1.89 ± 1.30^{d}	1.81 ± 1.31^{cd}	11.26 ± 1.28^{ae}
240	Normal (n=30,25,29)	6.50 ± 1.25^{d}	3.21 ± 1.28^{ae}	8.93 ± 1.26^{abf}
240	Propionic (n=27,29,27)	3.92 ± 1.28^{d}	8.76 ± 1.28^{bce}	7.77 ± 1.28^{be}
255	Acetic (n=24,24,26)	4.50 ± 1.33^{h}	5.43 ± 1.33^{abh}	7.24 ± 1.29^{bh}
255	Butyric (n=23,23,26)	2.25 ± 1.32^{dh}	1.90 ± 1.32^{bdh}	11.02 ± 1.29^{beh}
255	Normal (n=29,28,28)	6.20 ± 1.25^{deh}	6.20 ± 1.28^{adh}	8.63 ± 1.27^{abeh}
255	Propionic (n=27,24,27)	4.16 ± 1.28^{dh}	3.38 ± 1.29^{bdh}	7.51 ± 1.28^{beh}

SEM=standard error of the mean
Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

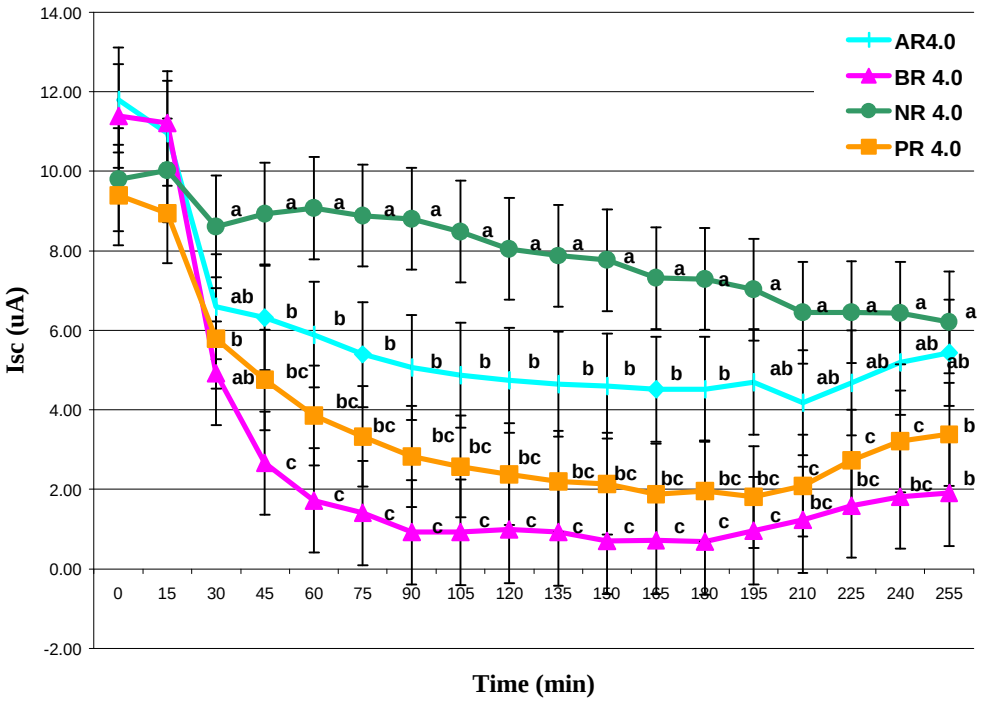


Figure 2-3. Mean Short-Circuit Current - pH 4.0

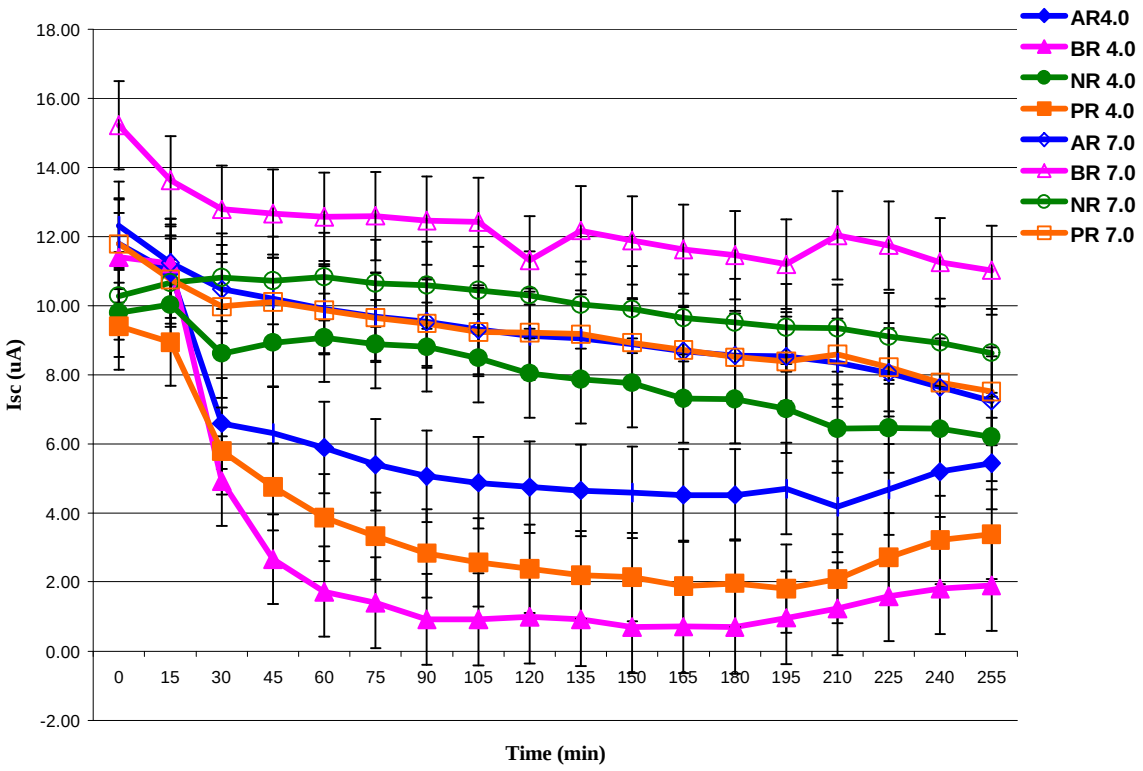


Figure 2-4. Mean Short-Circuit Current - pH 4.0 & pH 7.0

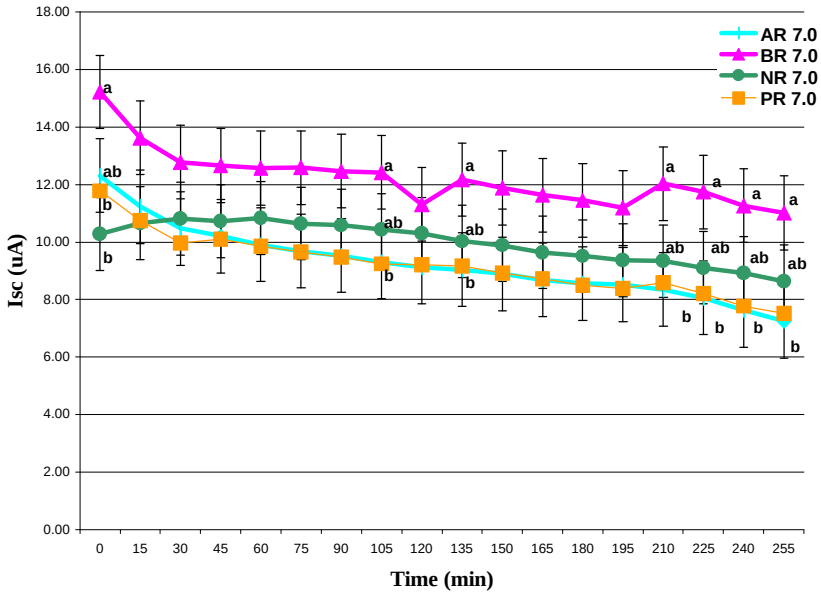


Figure 2-5. Mean Short-Circuit Current - pH 7.0

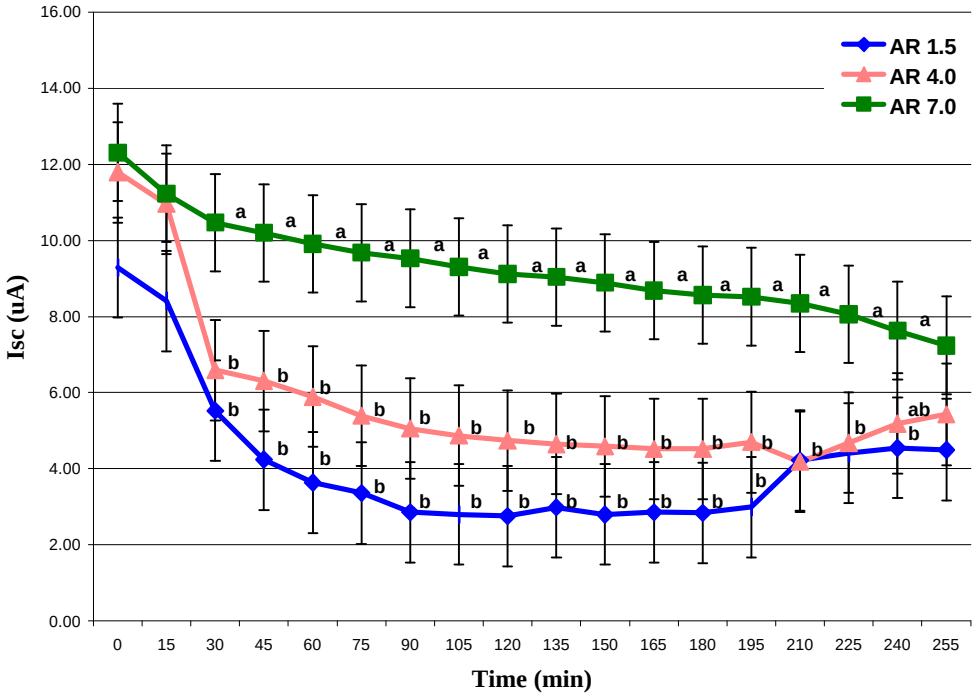


Figure 2-6. Mean Short-Circuit Current: Acetic Ringer's Solution

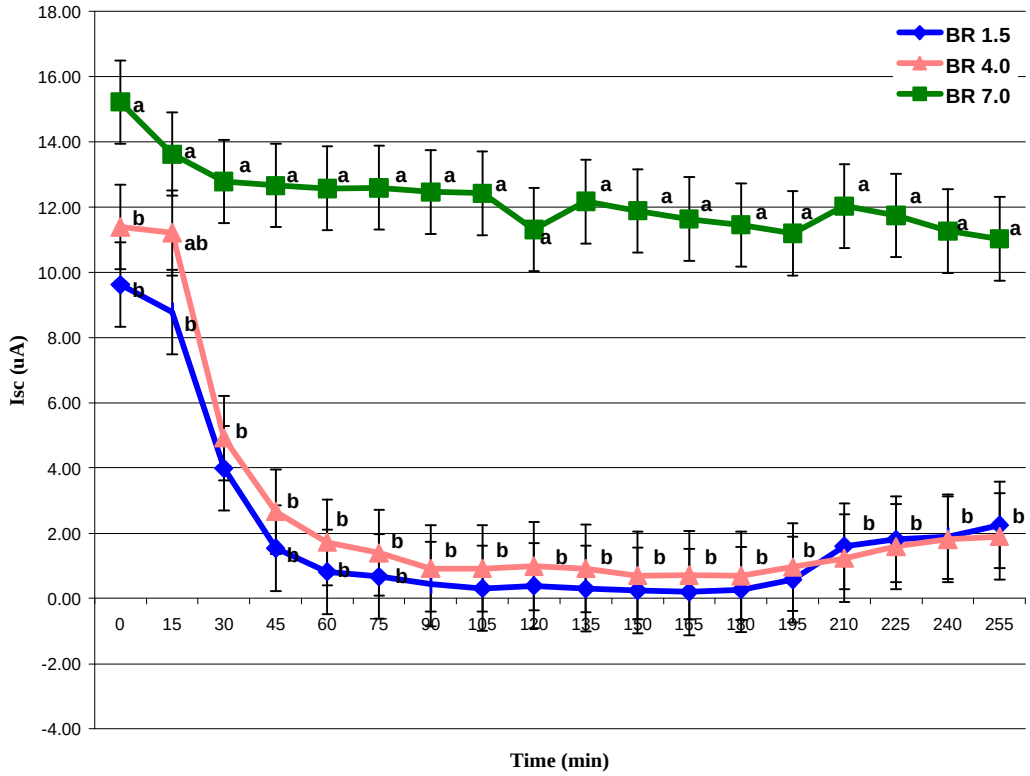


Figure 2-7. Mean Short-Circuit Current: Butyric Ringer's Solution

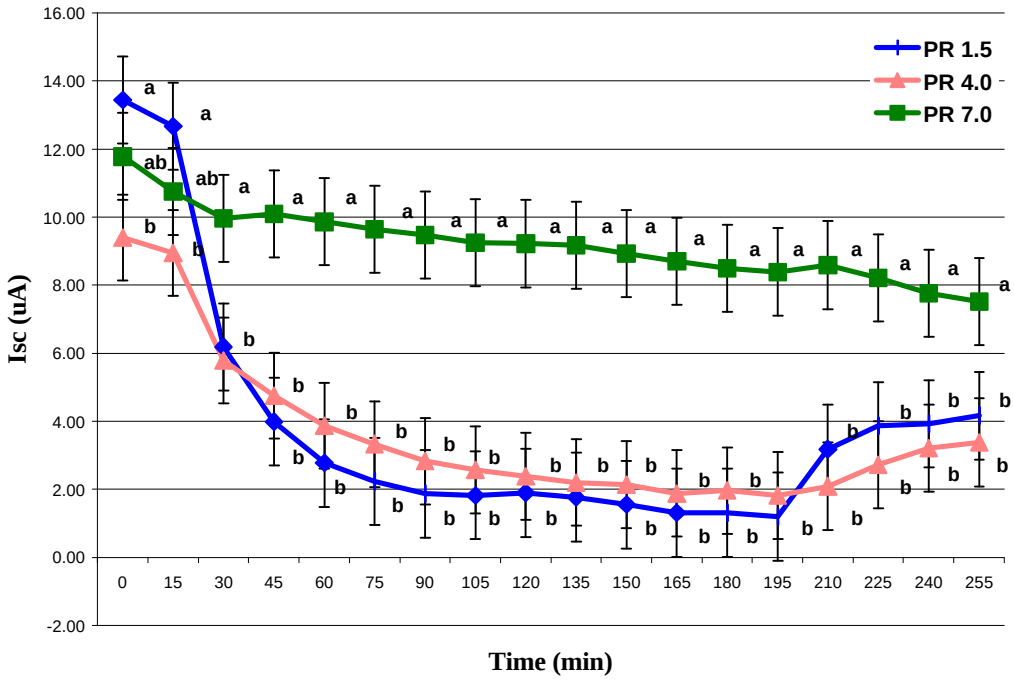


Figure 2-8. Mean Short-Circuit Current: Propionic Ringer's Solution

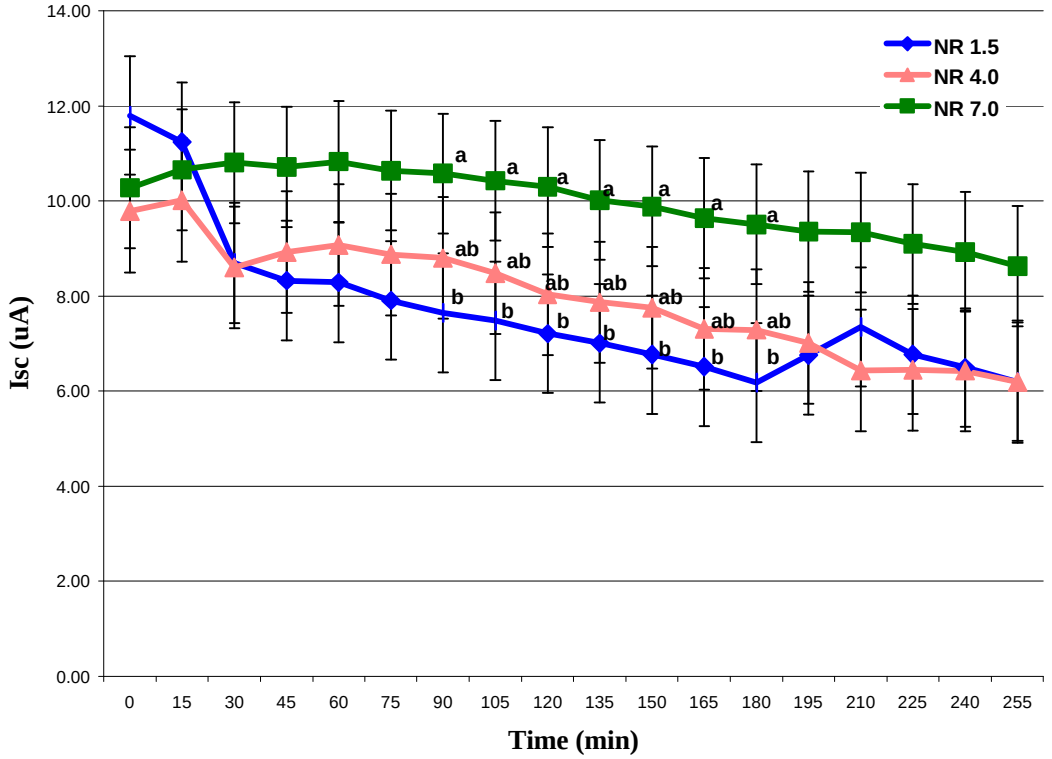


Figure 2-9. Mean Short-Circuit Current: Normal Ringer's Solution

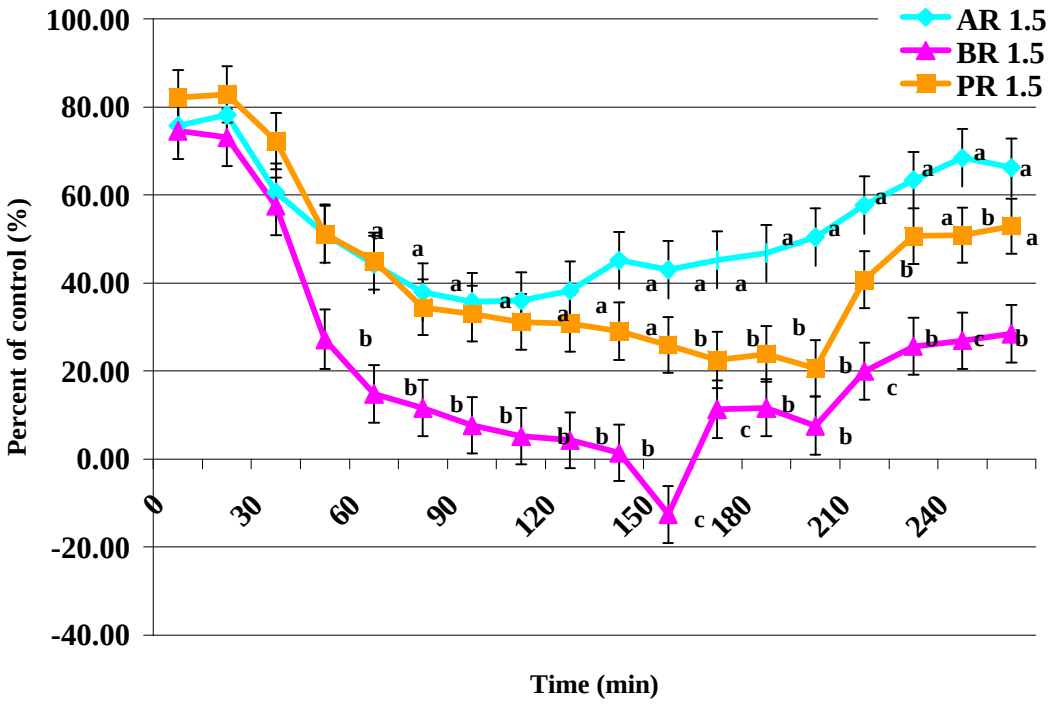


Figure 2-10. Mean Short-Circuit Current Percentage of Control pH 1.5

Table 2-3. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on percentage of short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (%) ±SEM @ pH 1.5	Mean (%) ±SEM @ pH 4.0	Mean (%) ±SEM @ pH 7.0
0	Acetic (n=25,22,27)	75.74 ± 6.50^{g}	87.96 ± 6.82^{g}	90.83 ± 6.34^{g}
0	Butyric (n=26,24,26)	74.56 ± 6.38^{g}	84.99 ± 6.62^{g}	90.77 ± 6.39^{g}
0	Propionic (n=28,26,26)	82.10 ± 6.23^{g}	82.93 ± 6.39^{g}	87.81 ± 6.39^{g}
15	Acetic (n=23,19,25)	78.14 ± 6.62	84.42 ± 7.04	89.40 ± 6.44
15	Butyric (n=25,22,24)	73.03 ± 6.44^{d}	83.31 ± 6.75^{d}	90.30 ± 6.49^{e}
15	Propionic (n=25,25,26)	82.81 ± 6.38	81.06 ± 6.56	86.56 ± 6.39
30	Acetic (n=24,22,25)	60.60 ± 6.56^{d}	79.42 ± 6.82^{e}	87.27 ± 6.44^{e}
30	Butyric (n=24,24,24)	57.37 ± 6.49^{d}	67.02 ± 6.62^{d}	88.83 ± 6.49^{e}
30	Propionic (n=25,26,25)	72.17 ± 6.38	75.75 ± 6.39	81.06 ± 6.44
45	Acetic (n=22,23,22)	51.09 ± 6.68^{ad}	73.58 ± 6.76^{ae}	85.86 ± 6.44^{d}
45	Butyric (n=20,24,25)	27.13 ± 6.77^{bd}	44.07 ± 6.63^{bd}	88.60 ± 6.44^{e}
45	Propionic (n=24,27,26)	51.09 ± 6.44^{ad}	59.96 ± 6.34^{ad}	83.17 ± 6.39^{ae}
60	Acetic (n=24,23,26)	44.16 ± 6.56^{ad}	69.74 ± 6.76^{ae}	85.92 ± 6.39^{e}
60	Butyric (n=23,22,26)	14.74 ± 6.55^{bd}	29.42 ± 6.75^{cd}	87.28 ± 6.39^{e}
60	Propionic (n=24,26,26)	44.94 ± 6.44^{ad}	50.13 ± 6.39^{bd}	82.88 ± 6.39^{e}
75	Acetic (n=23,24,27)	37.82 ± 6.62^{ad}	67.84 ± 6.70^{ae}	86.51 ± 6.34^{f}
75	Butyric (n=26,23,27)	11.56 ± 6.38^{bd}	23.65 ± 6.69^{cd}	88.15 ± 6.34^{e}
75	Propionic (n=27,27,27)	34.42 ± 6.28^{ad}	47.58 ± 6.34^{bd}	82.92 ± 6.34^{e}
90	Acetic (n=26,24,27)	35.73 ± 6.45^{ad}	67.03 ± 6.70^{ae}	86.68 ± 6.34^{f}
90	Butyric (n=26,21,27)	7.61 ± 6.38^{bd}	22.66 ± 6.83^{cd}	88.37 ± 6.34^{e}
90	Propionic (n=26,26,27)	32.98 ± 6.33^{ad}	46.29 ± 6.39^{bd}	82.61 ± 6.34^{e}
105	Acetic (n=26,24,27)	35.96 ± 6.45^{ad}	67.26 ± 6.70^{ae}	86.28 ± 6.34^{f}
105	Butyric (n=26,20,27)	5.10 ± 6.38^{bd}	21.86 ± 6.91^{cd}	88.63 ± 6.34^{e}
105	Propionic (n=25,24,26)	31.12 ± 6.38^{ad}	44.35 ± 6.50^{bd}	82.21 ± 6.39^{e}
120	Acetic (n=24,24,27)	38.22 ± 6.56^{ad}	69.10 ± 6.70^{ae}	86.42 ± 6.34^{f}
120	Butyric (n=26,18,26)	4.23 ± 6.39^{bd}	20.17 ± 7.08^{cd}	87.92 ± 6.39^{e}
120	Propionic (n=23,24,26)	30.81 ± 6.50^{ad}	46.83 ± 6.50^{bd}	82.28 ± 6.39^{e}
135	Acetic (n=25,24,27)	45.10 ± 6.50^{ad}	69.34 ± 6.70^{ae}	86.64 ± 6.34^{f}
135	Butyric (n=25,17,26)	1.36 ± 6.44^{cd}	20.89 ± 7.18^{ce}	88.24 ± 6.39^{f}
135	Propionic (n=23,25,26)	29.02 ± 6.50^{bd}	45.33 ± 6.45^{bd}	83.05 ± 6.39^{e}
150	Acetic (n=25,24,27)	42.99 ± 6.50^{ad}	69.84 ± 6.70^{ae}	86.54 ± 6.34^{e}
150	Butyric (n=24,20,26)	-12.61 ± 6.49^{cd}	21.39 ± 6.91^{ce}	87.93 ± 6.39^{f}
150	Propionic (n=26,24,26)	25.86 ± 6.33^{bd}	43.73 ± 6.50^{be}	82.48 ± 6.39^{f}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-3. (cont.) Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on the percentage of short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (%) ±SEM @ pH 1.5	Mean (%) ±SEM @ pH 4.0	Mean (%) ±SEM @ pH 7.0
165	Acetic (n=26,24,27)	45.17 ± 6.45^{ad}	70.72 ± 6.70^{ae}	86.23 ± 6.34^{e}
165	Butyric (n=23,18,26)	11.26 ± 6.55^{cd}	21.56 ± 7.08^{ce}	88.18 ± 6.39^{f}
165	Propionic (n=25,25,26)	22.48 ± 6.38^{bd}	43.51 ± 6.45^{be}	82.62 ± 6.39^{f}
180	Acetic (n=25,24,27)	46.71 ± 6.50^{ad}	71.21 ± 6.70^{ae}	86.39 ± 6.34^{e}
180	Butyric (n=25,18,26)	11.57 ± 6.44^{bd}	21.72 ± 7.08^{cd}	87.85 ± 6.39^{e}
180	Propionic (n=25,25,26)	23.82 ± 6.38 ^{bd}	43.47 ± 6.44^{be}	82.91 ± 6.39^{f}
195	Acetic (n=25,22,25)	50.37 ± 6.50^{ad}	71.73 ± 6.82^{ae}	86.67 ± 6.44^{e}
195	Butyric (n=22,17,23)	7.48 ± 6.62^{bd}	21.93 ± 7.18^{cd}	88.75 ± 6.55^{e}
195	Propionic (n=24,24,25)	20.59 ± 6.44^{bd}	45.36 ± 6.50^{be}	83.70 ± 6.44^{f}
210	Acetic (n=25,24,27)	57.62 ± 6.50^{ad}	71.82 ± 6.70^{ade}	85.24 ± 6.34^{d}
210	Butyric (n=24,19,26)	19.93 ± 6.49^{cd}	30.26 ± 6.99^{cd}	91.09 ± 6.39^{e}
210	Propionic (n=23,23,25)	40.67 ± 6.50^{bd}	50.19 ± 6.56^{bd}	85.72 ± 6.44^{e}
225	Acetic (n=26,24,27)	63.34 ± 6.45^{ad}	76.05 ± 6.70^{ade}	86.35 ± 6.34^{e}
225	Butyric (n=25,23,27)	25.58 ± 6.44^{bd}	34.63 ± 6.69^{cd}	91.15 ± 6.34^{e}
225	Propionic (n=26,23,26)	50.66 ± 6.33^{ad}	58.21 ± 6.56^{bd}	86.19 ± 6.39^{e}
240	Acetic (n=25,24,27)	68.39 ± 6.50^{ad}	79.30 ± 6.70^{ade}	85.24 ± 6.34^{e}
240	Butyric (n=27,23,27)	26.84 ± 6.34^{cd}	38.90 ± 6.69^{cd}	90.26 ± 6.34^{e}
240	Propionic (n=27,23,26)	50.79 ± 6.28^{bd}	61.66 ± 6.56^{bd}	85.11 ± 6.39^{e}
255	Acetic (n=24,24,25)	66.17 ± 6.56^{adg}	81.35 ± 6.70^{adeg}	84.47 ± 6.44^{aeg}
255	Butyric (n=23,22,25)	28.44 ± 6.55^{bdh}	41.90 ± 6.76^{cdh}	89.90 ± 6.44^{beg}
255	Propionic (n=27,24,26)	52.91 ± 6.28^{adh}	62.70 ± 6.50^{bdh}	85.18 ± 6.39^{aeg}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

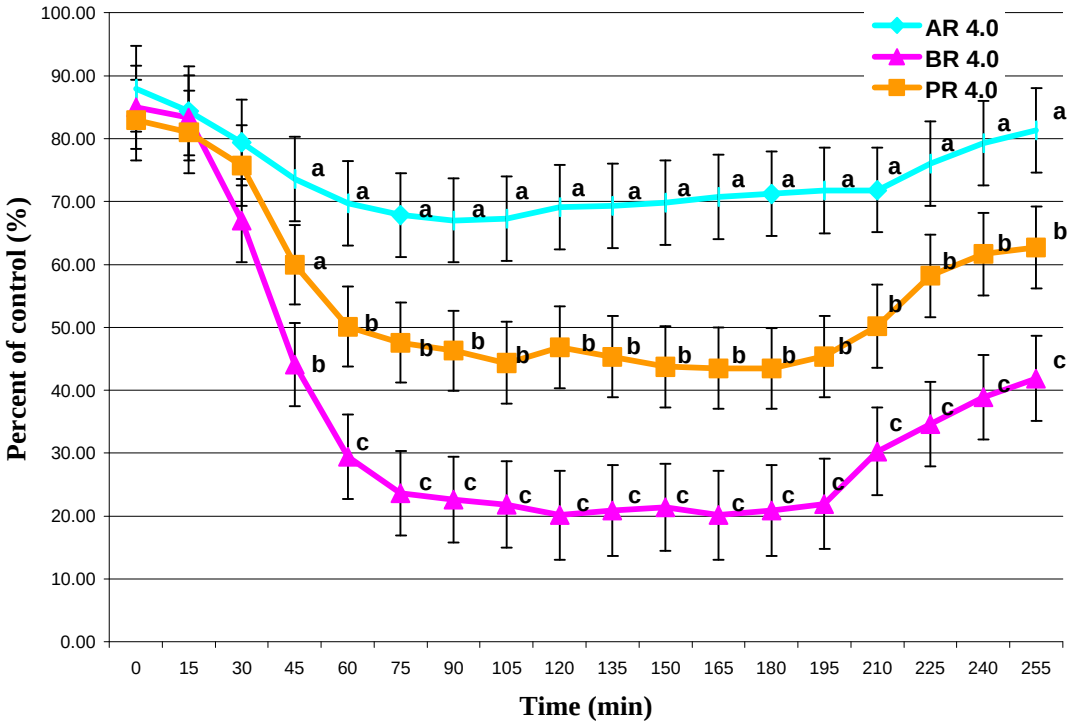


Figure 2-11. Mean Short-Circuit Current Percentage of Control pH 4.0

There were no significant ($P<0.05$) differences in PCIsc values at pH 7.0 (Table 2-3, Figure 2-12).

Acetic acid decreased ($P<0.01$) Isc the greatest at pH 1.5 followed by pH 4.0 and pH 7.0 (Figure 2-13). Butyric acid decreased ($P<0.01$) PCIsc the greatest at pH 1.5 and 4.0 compared to pH 7.0 (Figure 2-14). Propionic acid decreased ($P<0.01$) PCIsc from 135-195 min at pH 1.5 compared to pH 4.0 or pH 7.0 (Figure 2-15).

Butyric acid and propionic acid decreased ($P<0.01$) PD following the 30 minute acclimation period at pH 1.5 (Figure 2-16, Table 2-4). In each case, there was some, but not complete recovery following 195 min of exposure.

Butyric acid and propionic acid decreased ($P<0.01$) PD following the 30 minute acclimation period at pH 4.0 (Figure 2-17, Table 2-4). In each case, there was some, but not complete recovery following 195 min of exposure. PD was decreased the greatest with butyric acid exposure followed by propionic acid.

There were no significant differences in PD at pH 7.0 (Figure 2-18, Table 2-24). Acetic acid, butyric acid, and normal Ringer's solution decreased ($P<0.01$) PD at pH 1.5 compared to pH 4.0 and 7.0 (Figure 2-19, 2-20, 2-21). Propionic acid decreased ($P<0.01$) PD at pH 1.5 followed by pH 4.0 and pH 7.0 (Figure 2-22).

Butyric acid increased ($P<0.01$) R following the 30 min acclimation period at pH 1.5 and 4.0. There was some, but not complete, recovery following 195 min of exposure at both pHs (Figure 2-23 and 2-24, Table 2-5).

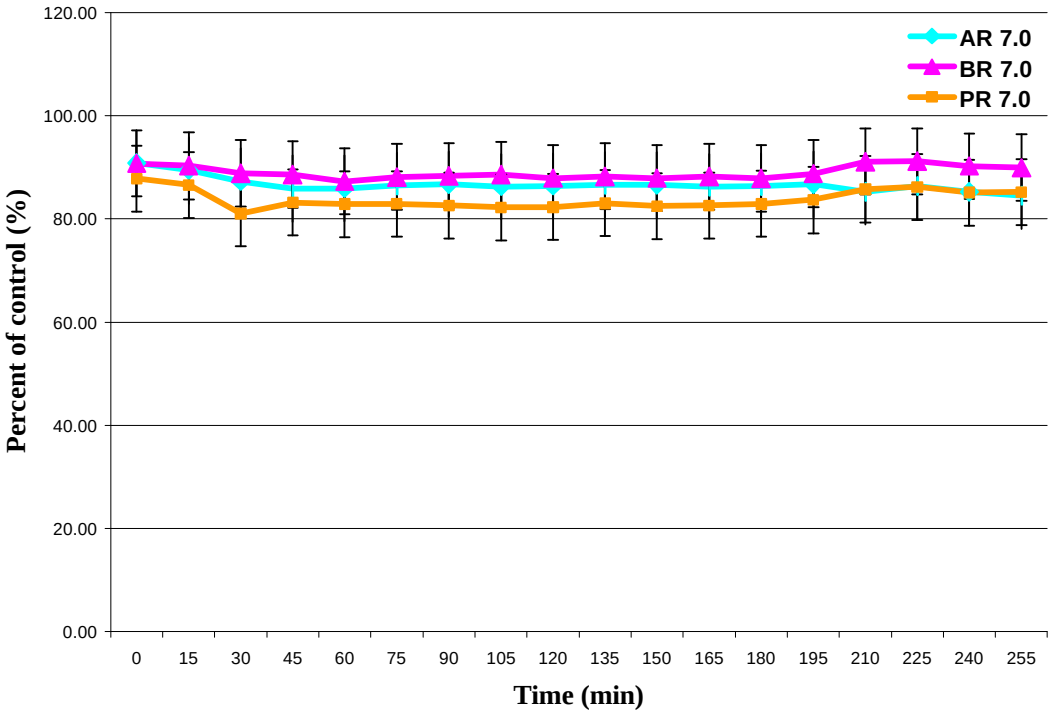


Figure 2-12. Mean Short-Circuit Current Percentage of Control pH 7.0

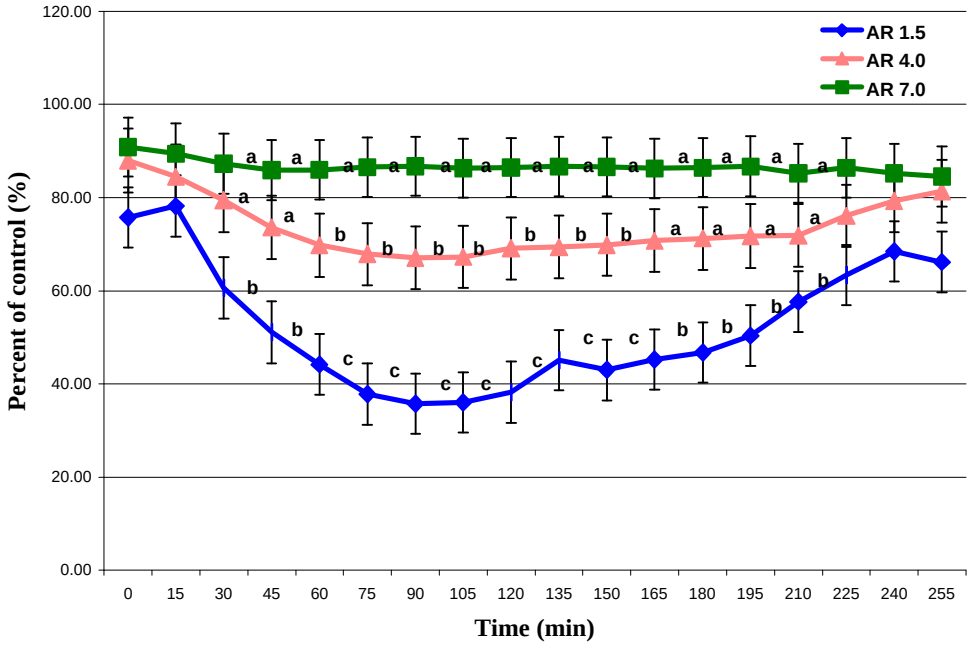


Figure 2-13. Mean Short-Circuit Percentage of Control: Acetic Ringer's Solution

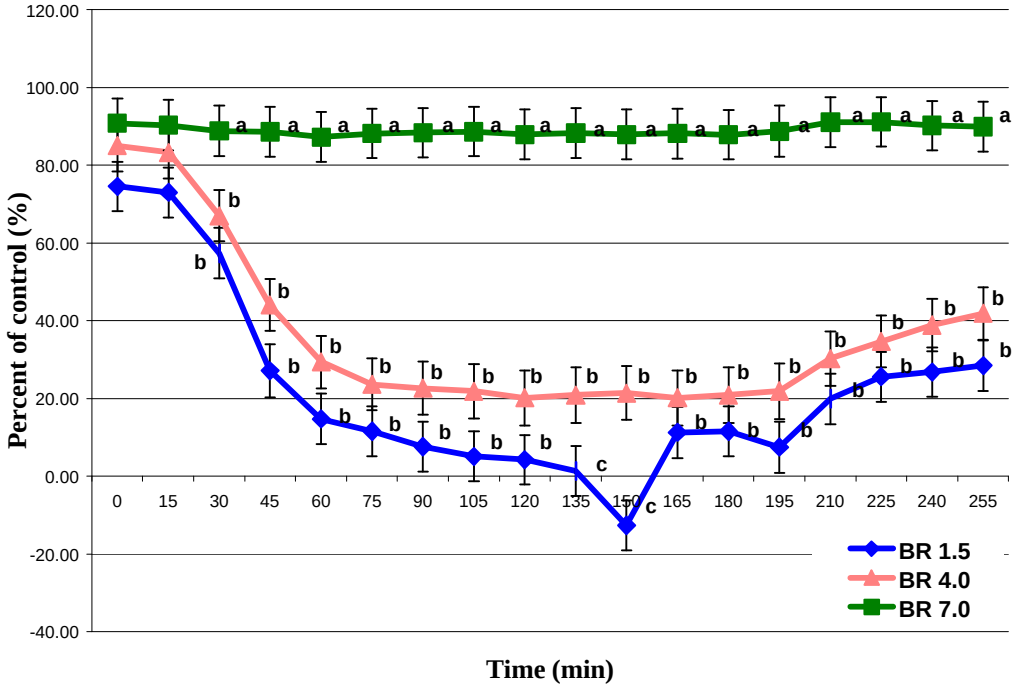


Figure 2-14. Mean Short-Circuit Current Percentage of Control: Butyric Ringer's Solution

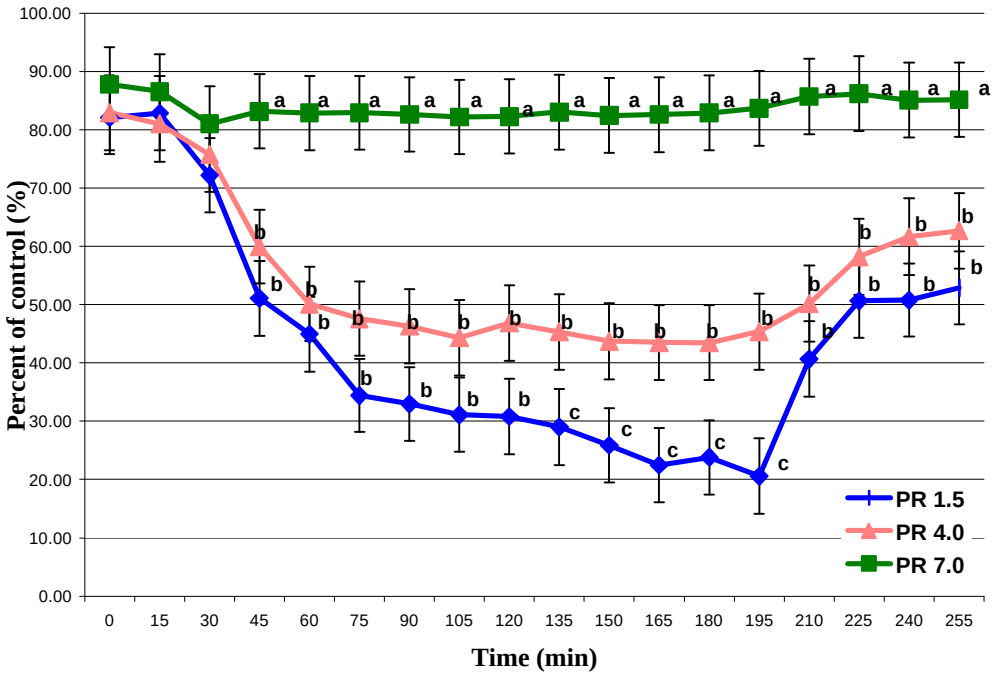


Figure 2-15. Mean Short-Circuit Current Percentage of Control: Propionic Ringer's Solution

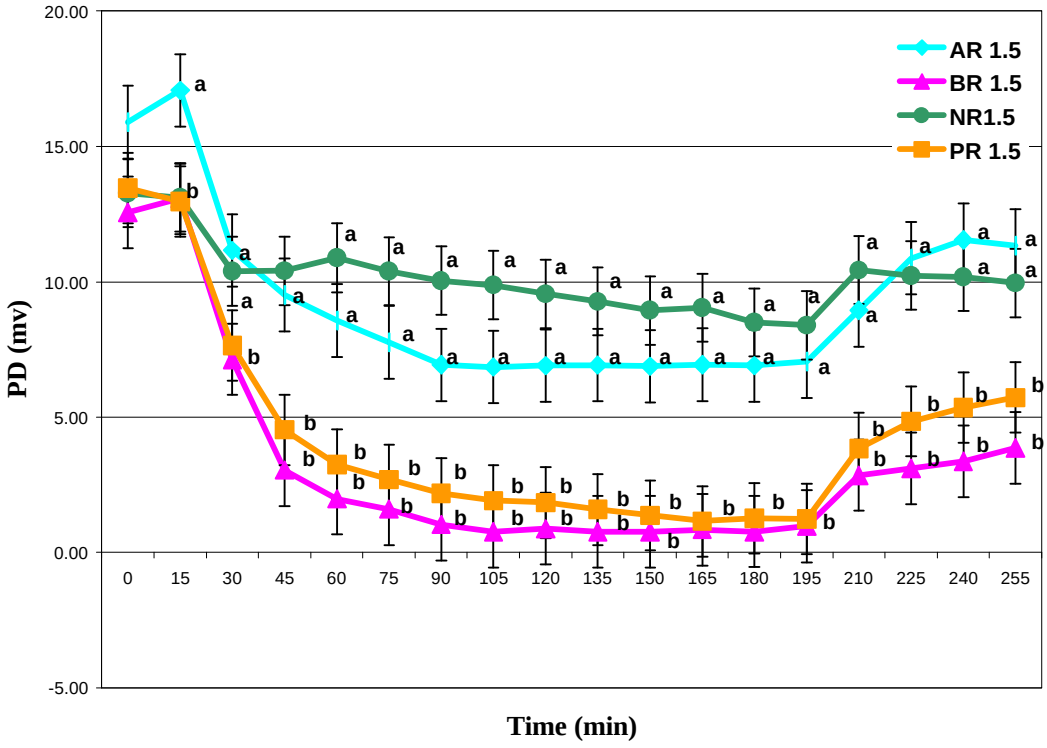


Figure 2-16. Mean Potential Difference - pH 1.5

Table 2-4. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mv) ±SEM @ pH 1.5	Mean (mv) ±SEM @ pH 4.0	Mean (mv) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	15.90 ± 1.35^{g}	15.88 ± 1.35^{g}	13.78 ± 1.30^{g}
0	Butyric (n=26,26,27)	12.56 ± 1.32^{g}	13.50 ± 1.32^{g}	15.54 ± 1.30^{g}
0	Normal (n=29,27,28)	13.27 ± 1.26^{g}	13.95 ± 1.30^{g}	12.63 ± 1.28^{g}
0	Propionic (n=28,29,28)	13.46 ± 1.30^{g}	13.95 ± 1.28^{g}	13.46 ± 1.30^{g}
15	Acetic (n=26,25,28)	17.07 ± 1.34^{ad}	15.00 ± 1.35^{de}	13.10 ± 1.30^{e}
15	Butyric (n=27,27,27)	13.07 ± 1.32^{b}	13.91 ± 1.32	15.16 ± 1.30
15	Normal (n=29,27,29)	13.11 ± 1.26^{b}	14.05 ± 1.30	12.75 ± 1.28
15	Propionic (n=28,29,28)	12.97 ± 1.30^{b}	13.51 ± 1.28	13.37 ± 1.30
30	Acetic (n=26,25,28)	11.16 ± 1.34^{a}	11.14 ± 1.35	13.66 ± 1.30
30	Butyric (n=26,27,27)	7.13 ± 1.32^{bd}	8.40 ± 1.32^{d}	14.75 ± 1.30^{e}
30	Normal (n=28,27,29)	10.39 ± 1.27^{a}	13.12 ± 1.30	13.61 ± 1.28
30	Propionic (n=27,29,27)	7.64 ± 1.30^{bd}	10.55 ± 1.28^{de}	12.85 ± 1.30^{e}
45	Acetic (n=25,25,27)	9.51 ± 1.35^{ad}	11.92 ± 1.35^{ade}	13.72 ± 1.30^{e}
45	Butyric (n=25,27,28)	3.04 ± 1.33^{bd}	5.86 ± 1.32^{bd}	14.75 ± 1.30^{e}
45	Normal (n=29,28,29)	10.40 ± 1.26^{ad}	13.89 ± 1.30^{ae}	13.67 ± 1.28^{e}
45	Propionic (n=28,29,28)	4.53 ± 1.30^{bd}	9.53 ± 1.28^{ae}	13.30 ± 1.30^{f}
60	Acetic (n=26,26,28)	8.57 ± 1.34^{ad}	12.04 ± 1.34^{ae}	13.85 ± 1.30^{e}
60	Butyric (n=26,27,28)	1.98 ± 1.32^{bd}	3.76 ± 1.32^{cd}	14.95 ± 1.30^{e}
60	Normal (n=27,27,29)	10.88 ± 1.27^{ad}	14.56 ± 1.30^{ae}	13.60 ± 1.28^{de}
60	Propionic (n=28,29,28)	3.25 ± 1.30^{bd}	8.07 ± 1.28^{be}	12.99 ± 1.30^{f}
75	Acetic (n=25,26,28)	7.76 ± 1.35^{ad}	11.88 ± 1.34^{ae}	14.00 ± 1.30^{e}
75	Butyric (n=27,27,28)	1.60 ± 1.32^{bd}	2.86 ± 1.32^{cd}	15.25 ± 1.30^{e}
75	Normal (n=30,28,29)	10.39 ± 1.26^{ad}	14.68 ± 1.30^{ae}	13.93 ± 1.28^{e}
75	Propionic (n=27,29,28)	2.69 ± 1.30^{bd}	7.42 ± 1.28^{be}	13.13 ± 1.30^{f}
90	Acetic (n=26,26,28)	6.93 ± 1.34^{ad}	12.11 ± 1.34^{ae}	14.14 ± 1.30^{e}
90	Butyric (n=27,27,28)	1.03 ± 1.32^{bd}	2.83 ± 1.32^{cd}	15.30 ± 1.30^{e}
90	Normal (n=30,28,29)	10.04 ± 1.26^{ad}	14.67 ± 1.30^{ae}	14.15 ± 1.28^{e}
90	Propionic (n=27,29,28)	2.18 ± 1.30^{bd}	7.03 ± 1.28^{be}	13.07 ± 1.30^{f}
105	Acetic (n=26,26,28)	6.85 ± 1.34^{ad}	11.59 ± 1.34^{ae}	14.10 ± 1.30^{e}
105	Butyric (n=26,27,28)	0.76 ± 1.32^{bd}	2.05 ± 1.32^{cd}	15.52 ± 1.30^{e}
105	Normal (n=30,28,29)	9.88 ± 1.26^{ad}	14.53 ± 1.30^{ae}	14.37 ± 1.28^{e}
105	Propionic (n=25,29,28)	1.91 ± 1.31^{bd}	6.68 ± 1.28^{be}	12.98 ± 1.30^{f}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-4 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mv) ±SEM @ pH 1.5	Mean (mv) ±SEM @ pH 4.0	Mean (mv) ±SEM @ pH 7.0
120	Acetic (n=26,26,28)	6.90 ± 1.34^{ad}	11.67 ± 1.34^{ae}	14.10 ± 1.30^{e}
120	Butyric (n=26,26,27)	0.87 ± 1.32^{bd}	1.80 ± 1.32^{cd}	15.51 ± 1.30^{e}
120	Normal (n=30,28,29)	9.55 ± 1.26^{ad}	14.39 ± 1.30^{ae}	14.69 ± 1.28^{e}
120	Propionic (n=26,29,28)	1.84 ± 1.31^{bd}	6.66 ± 1.28^{be}	12.58 ± 1.30^{f}
135	Acetic (n=26,26,28)	6.92 ± 1.34^{ad}	12.11 ± 1.34^{ae}	14.22 ± 1.30^{e}
135	Butyric (n=26,26,27)	0.76 ± 1.32^{bd}	1.69 ± 1.32^{cd}	15.58 ± 1.30^{e}
135	Normal (n=30,28,29)	9.27 ± 1.26^{ad}	14.23 ± 1.30^{ae}	14.73 ± 1.28^{e}
135	Propionic (n=26,29,28)	1.59 ± 1.31^{bd}	6.50 ± 1.28^{be}	12.13 ± 1.30^{f}
150	Acetic (n=26,26,28)	6.88 ± 1.34^{ad}	11.81 ± 1.34^{ae}	14.22 ± 1.30^{e}
150	Butyric (n=26,27,27)	0.76 ± 1.32^{bd}	1.68 ± 1.32^{cd}	15.66 ± 1.30^{e}
150	Normal (n=30,28,29)	8.94 ± 1.26^{ad}	14.11 ± 1.30^{ae}	14.77 ± 1.28^{e}
150	Propionic (n=27,29,28)	1.37 ± 1.30^{bd}	6.32 ± 1.28^{be}	12.05 ± 1.30^{f}
165	Acetic (n=26,26,28)	6.94 ± 1.34^{ad}	11.93 ± 1.34^{ae}	14.15 ± 1.30^{e}
165	Butyric (n=26,27,27)	0.84 ± 1.32^{bd}	1.69 ± 1.32^{cd}	15.58 ± 1.30^{e}
165	Normal (n=30,28,29)	9.04 ± 1.26^{ad}	13.63 ± 1.30^{ae}	14.75 ± 1.28^{e}
165	Propionic (n=27,29,28)	1.15 ± 1.30^{bd}	6.04 ± 1.28^{be}	11.91 ± 1.30^{f}
180	Acetic (n=26,26,28)	6.90 ± 1.34^{ad}	12.07 ± 1.34^{ae}	14.13 ± 1.30^{e}
180	Butyric (n=26,27,27)	0.77 ± 1.32^{bd}	1.62 ± 1.32^{cd}	15.52 ± 1.30^{e}
180	Normal (n=30,28,29)	8.50 ± 1.26^{ad}	13.79 ± 1.30^{ae}	14.77 ± 1.28^{e}
180	Propionic (n=26,29,28)	1.26 ± 1.31^{bd}	5.92 ± 1.28^{be}	11.80 ± 1.30^{f}
195	Acetic (n=25,24,26)	7.06 ± 1.35^{ad}	11.97 ± 1.35^{ae}	14.19 ± 1.31^{e}
195	Butyric (n=23,25,24)	0.96 ± 1.34^{bd}	1.76 ± 1.33^{cd}	15.48 ± 1.32^{e}
195	Normal (n=29,27,28)	8.39 ± 1.26^{ad}	13.52 ± 1.30^{ae}	14.67 ± 1.28^{e}
195	Propionic (n=27,28,27)	1.23 ± 1.30^{bd}	5.96 ± 1.28^{be}	11.71 ± 1.30^{f}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-4 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mv) ±SEM @ pH 1.5	Mean (mv) ±SEM @ pH 4.0	Mean (mv) ±SEM @ pH 7.0
210	Acetic (n=26,26,28)	8.95 ± 1.34^{ad}	10.83 ± 1.34^{ade}	13.78 ± 1.30^{e}
210	Butyric (n=26,27,27)	2.85 ± 1.32^{bd}	2.05 ± 1.32^{cd}	15.44 ± 1.30^{e}
210	Normal (n=30,28,29)	10.43 ± 1.26^{ad}	12.80 ± 1.30^{ade}	14.40 ± 1.28^{e}
210	Propionic (n=26,29,28)	3.84 ± 1.31^{bd}	6.36 ± 1.28^{bd}	11.39 ± 1.30^{e}
225	Acetic (n=26,26,28)	10.86 ± 1.34^{ad}	12.25 ± 1.34^{a}	13.13 ± 1.30
225	Butyric (n=26,27,28)	3.10 ± 1.32^{bd}	2.82 ± 1.32^{cd}	15.25 ± 1.30^{e}
225	Normal (n=30,28,29)	10.23 ± 1.26^{ad}	12.98 ± 1.30^{ade}	14.37 ± 1.28^{e}
225	Propionic (n=27,29,28)	4.84 ± 1.30^{bd}	7.81 ± 1.28^{bde}	10.88 ± 1.30^{e}
240	Acetic (n=25,26,28)	11.55 ± 1.35^{ad}	13.32 ± 1.34^{a}	12.89 ± 1.30
240	Butyric (n=27,27,28)	3.37 ± 1.32^{bd}	3.29 ± 1.32^{cd}	14.76 ± 1.30^{e}
240	Normal (n=30,28,29)	10.17 ± 1.26^{ad}	12.86 ± 1.30^{ade}	14.15 ± 1.28^{e}
240	Propionic (n=27,29,28)	5.35 ± 1.30^{bd}	8.76 ± 1.28^{be}	10.38 ± 1.30^{e}
255	Acetic (n=24,24,26)	11.33 ± 1.35^{ah}	13.53 ± 1.35^{ag}	12.21 ± 1.31^{h}
255	Butyric (n=25,26,26)	3.86 ± 1.33^{bdh}	5.10 ± 1.32^{cdh}	14.59 ± 1.31^{eg}
255	Normal (n=29,28,28)	9.95 ± 1.26^{adh}	12.62 ± 1.30^{adeg}	13.87 ± 1.28^{eg}
255	Propionic (n=27,27,27)	5.72 ± 1.30^{bdh}	9.44 ± 1.29^{beh}	10.48 ± 1.30^{eh}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

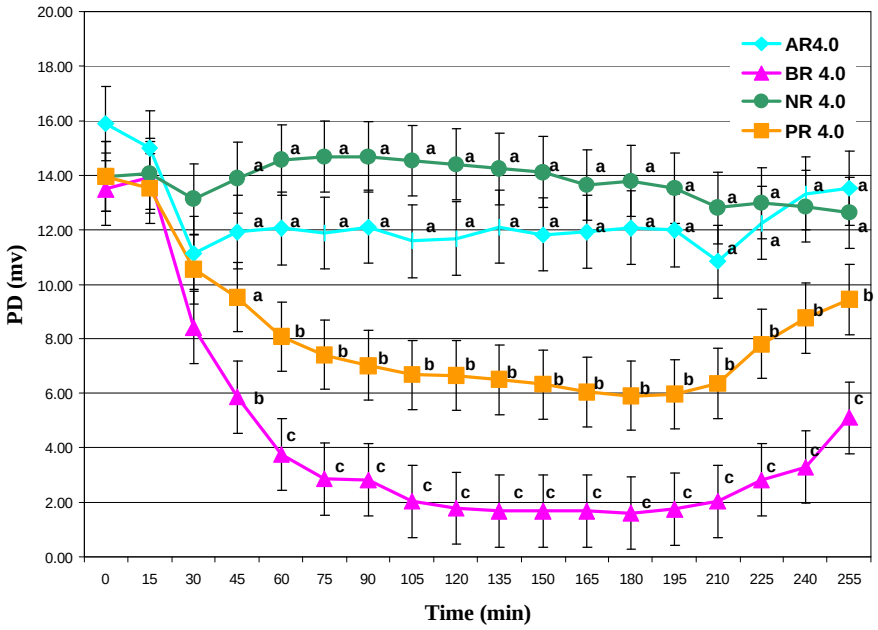


Figure 2-17. Mean Potential Difference - pH 4.0

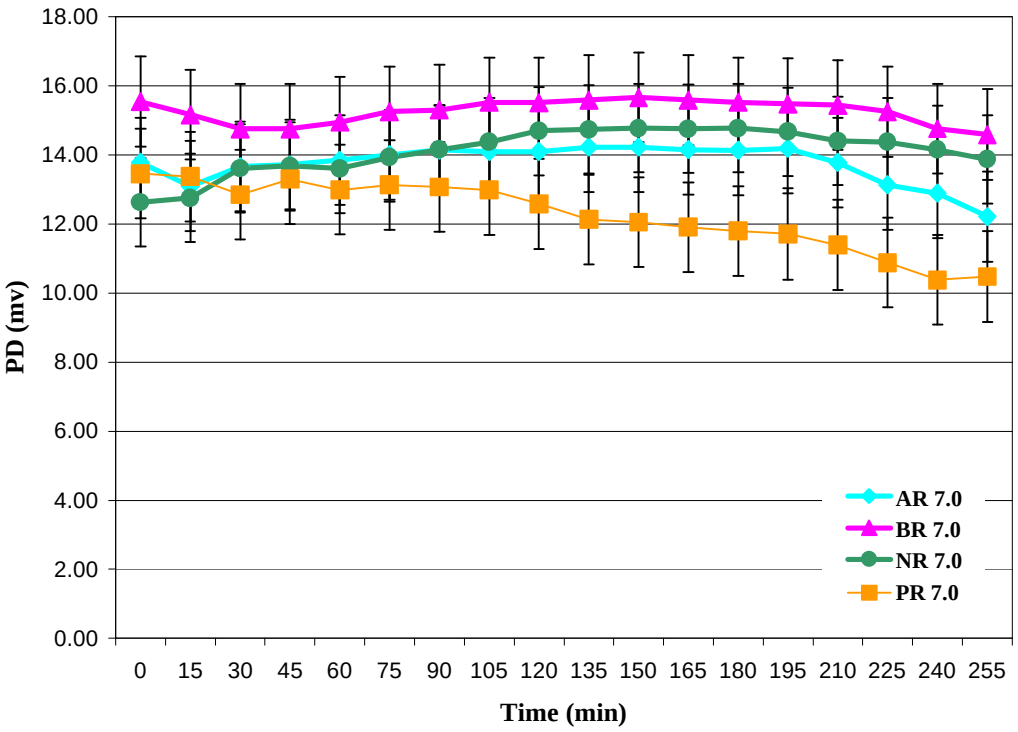


Figure 2-18. Mean Potential Difference - pH 7.0

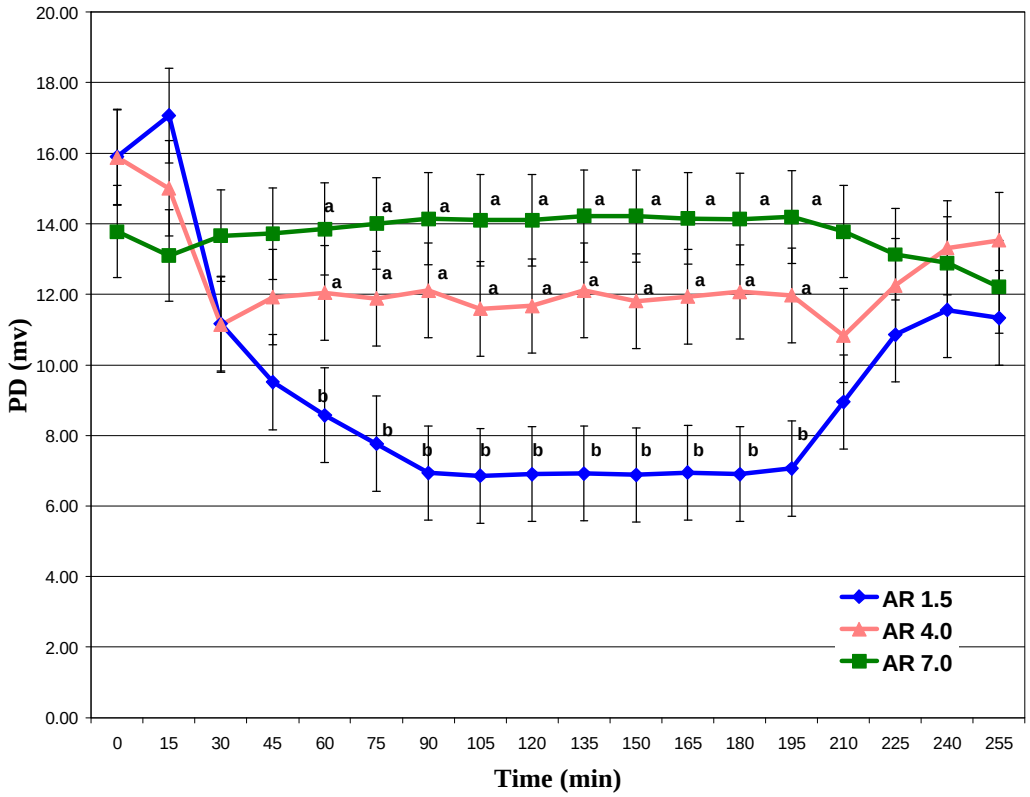


Figure 2-19. Mean PD: Acetic Ringer's Solution

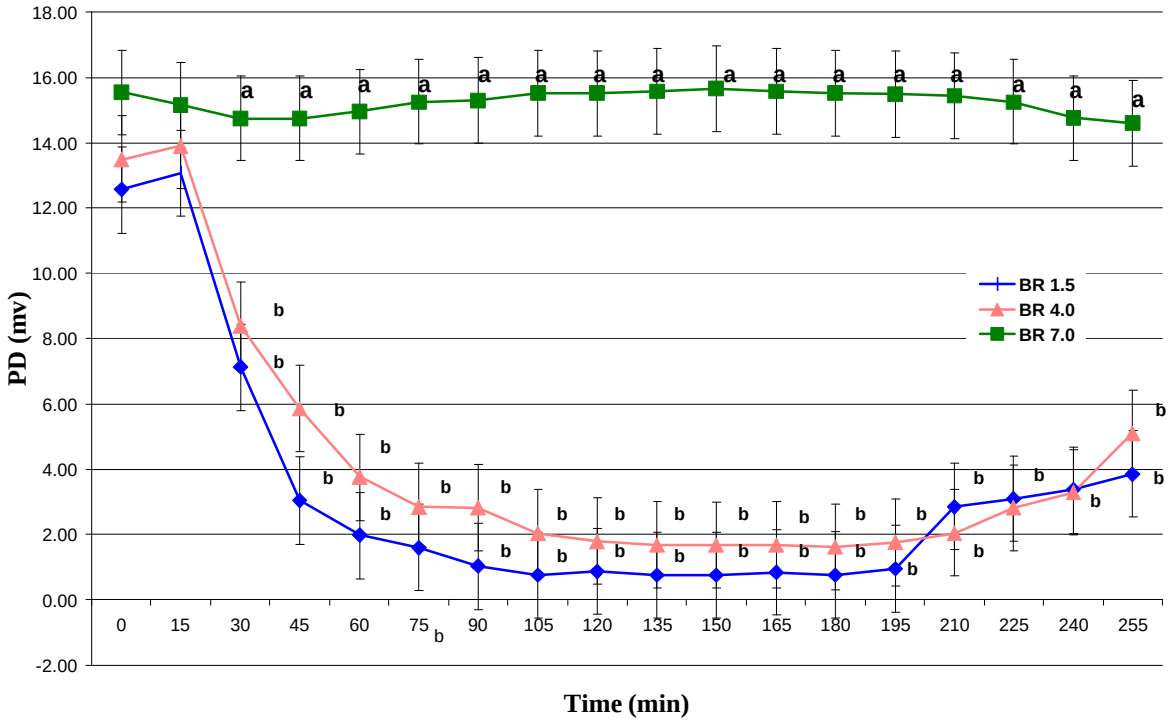


Figure 2-20. Mean PD: Butyric Ringer's Solution

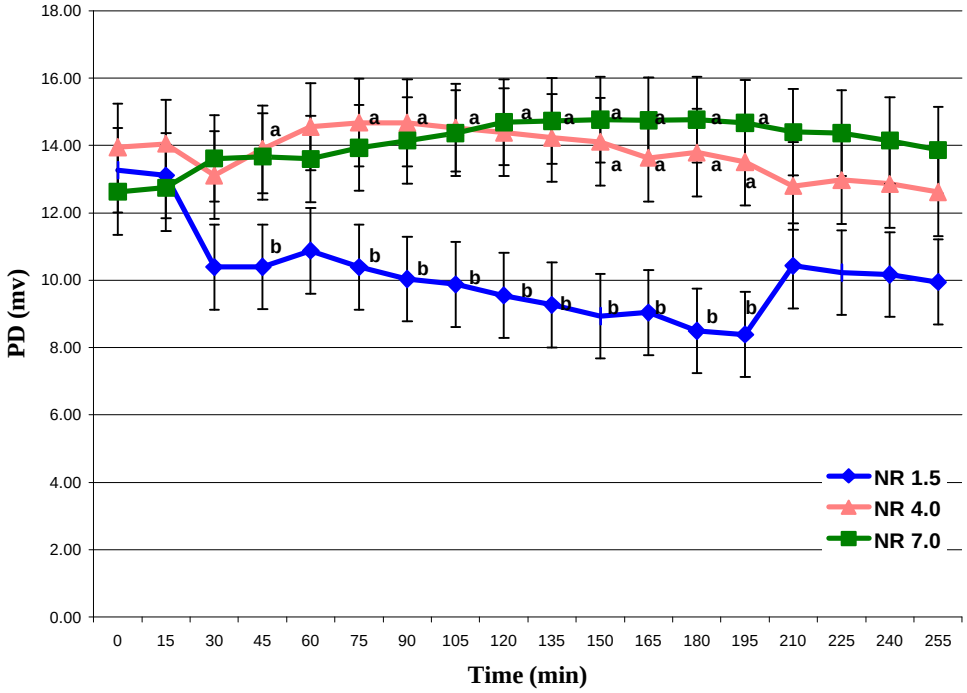


Figure 2-21. Mean PD: Normal Ringer's Solution

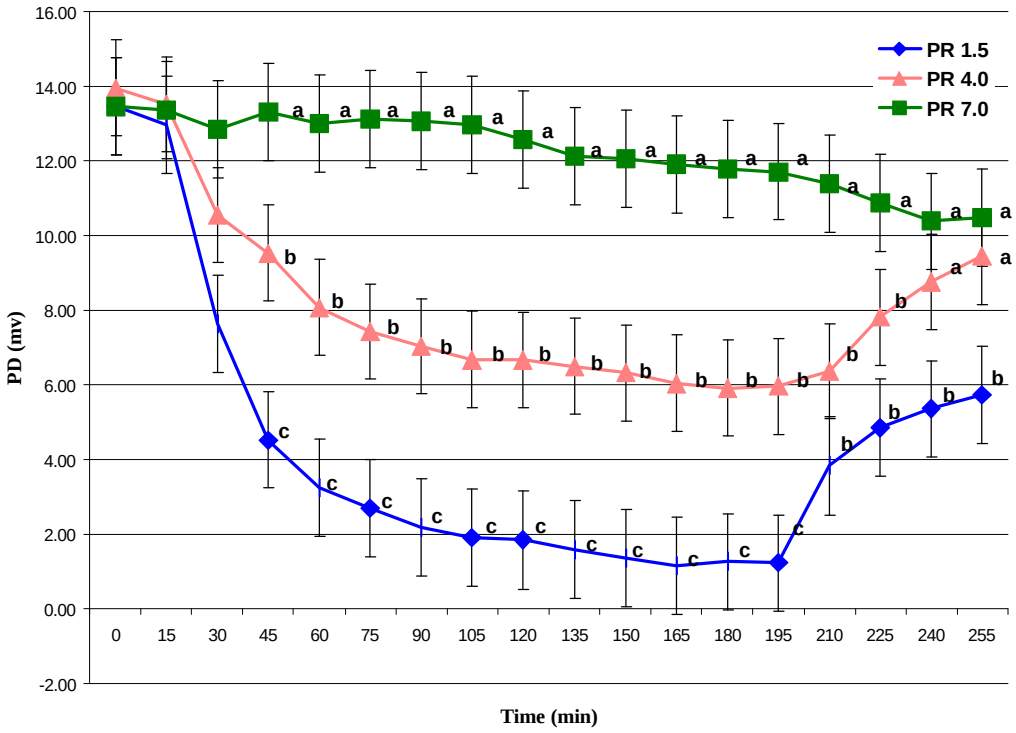


Figure 2-22. Mean PD: Propionic Ringer's Solution

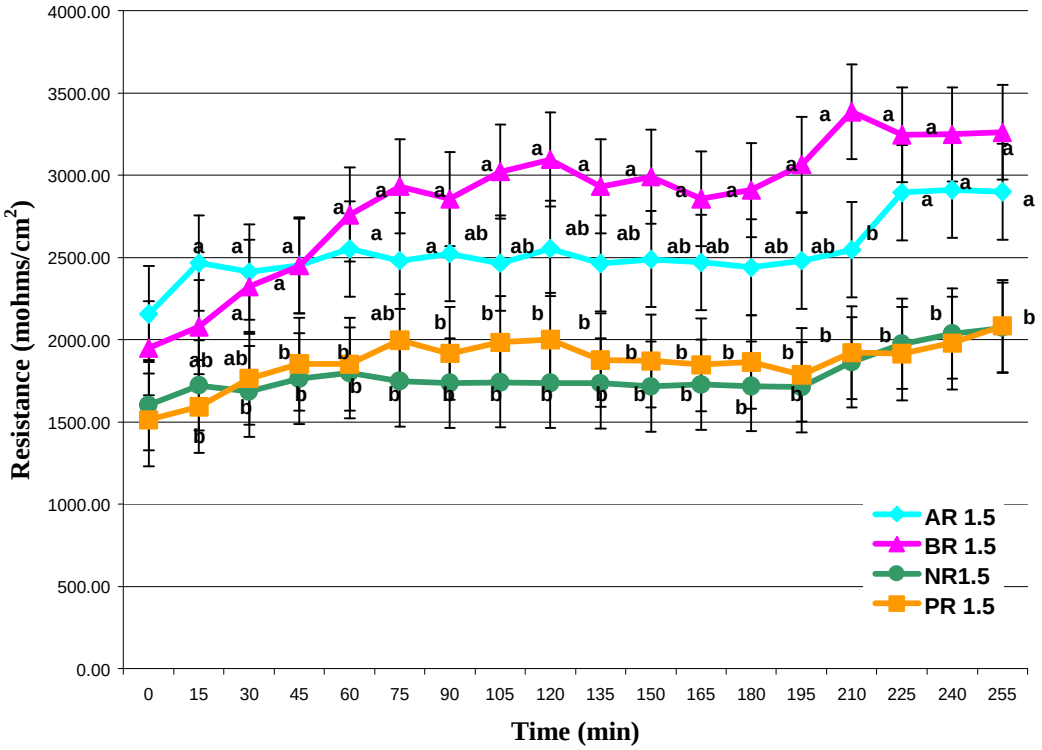


Figure 2-23. Mean Resistance - pH 1.5

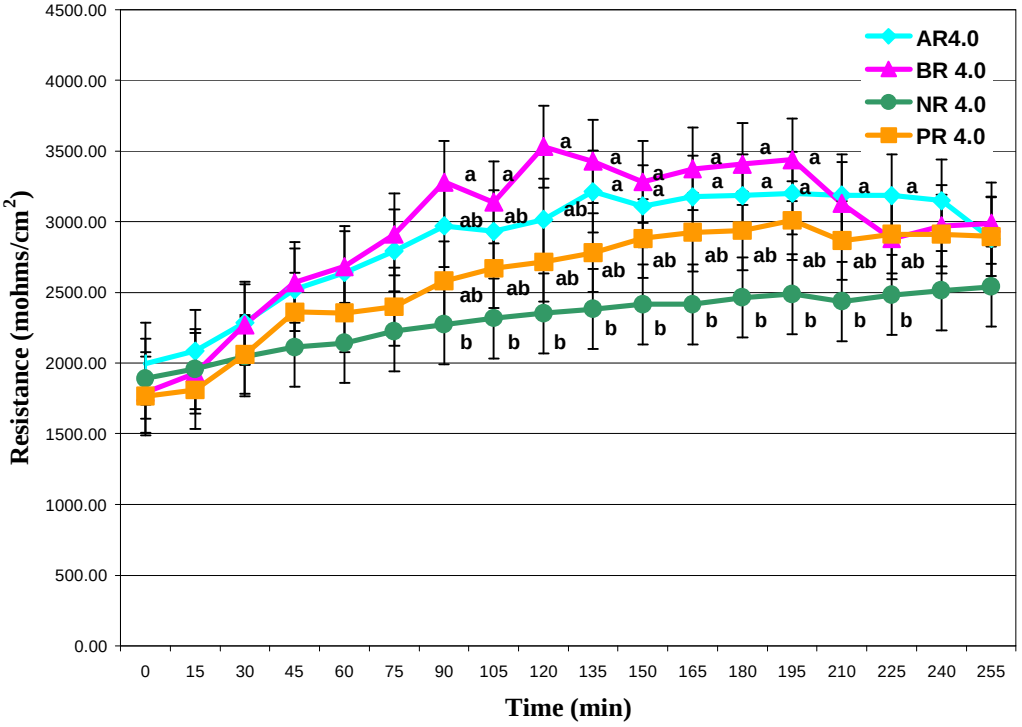


Figure 2-24. Mean Resistance - pH 4.0

Table 2-5. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	2156.63 ± 291.16^{g}	1996.36 ± 291.13^{g}	1651.72 ± 282.41^{g}
0	Butyric (n=26,26,27)	1949.40 ± 286.46^{g}	1792.72 ± 286.56^{g}	1758.35 ± 282.32^{g}
0	Normal (n=29,26,27)	1602.27 ± 274.41^{g}	1890.15 ± 282.66^{g}	1972.16 ± 278.62^{g}
0	Propionic (n=28,29,27)	1512.64 ± 281.91^{g}	1767.13 ± 277.92^{g}	1561.00 ± 282.31^{g}
15	Acetic (n=26,25,28)	2466.72 ± 290.69^{a}	2085.20 ± 291.13	1693.70 ± 281.99
15	Butyric (n=27,27,27)	2079.07 ± 286.01^{a}	1928.64 ± 286.11	1844.13 ± 282.32
15	Normal (n=27,24,27)	1722.68 ± 275.24^{ab}	1956.91 ± 283.67	2063.35 ± 278.62
15	Propionic (n=28,29,28)	1593.18 ± 281.91^{b}	1810.41 ± 277.92	1685.07 ± 281.90
30	Acetic (n=26,25,28)	2412.00 ± 290.69^{a}	2283.10 ± 291.13	1825.34 ± 281.99
30	Butyric (n=26,27,27)	2322.68 ± 286.46^{a}	2270.32 ± 286.11	1989.01 ± 282.32
30	Normal (n=28,27,28)	1686.61 ± 274.81^{b}	2045.84 ± 282.20	2188.58 ± 278.19
30	Propionic (n=27,29,27)	1764.68 ± 282.33^{ab}	2062.18 ± 277.92	1752.42 ± 282.31
45	Acetic (n=25,25,27)	2453.49 ± 291.16^{a}	2519.14 ± 291.12	1881.54 ± 282.41
45	Butyric (n=23,26,27)	2450.08 ± 288.04^{ab}	2569.54 ± 286.56	2047.83 ± 282.32
45	Normal (n=27,28,29)	1763.78 ± 275.24^{b}	2112.76 ± 281.78	2146.64 ± 277.79
45	Propionic (n=27,29,28)	1853.32 ± 282.35^{b}	2360.04 ± 277.92	1804.07 ± 281.90
60	Acetic (n=26,26,28)	2552.18 ± 290.69^{a}	2639.86 ± 290.65	1950.98 ± 281.99
60	Butyric (n=26,25,28)	2761.32 ± 286.46^{a}	2683.13 ± 287.07	2102.60 ± 281.90
60	Normal (n=27,27,27)	1798.51 ± 275.24^{b}	2142.93 ± 282.20	2148.66 ± 278.62
60	Propionic (n=26,29,28)	1851.27 ± 282.80^{b}	2353.16 ± 277.92	1812.24 ± 281.90

SEM=standard error of the mean; Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-5 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
75	Acetic (n=23,26,28)	2481.81 ± 292.23^{a}	2792.81 ± 290.65	2005.74 ± 281.99
75	Butyric (n=26,25,28)	2932.82 ± 286.45^{ad}	2909.84 ± 287.09^{d}	2132.92 ± 281.90^{e}
75	Normal (n=30,28,29)	1748.07 ± 274.03^{b}	2225.60 ± 281.78	2129.69 ± 277.79
75	Propionic (n=27,29,28)	1997.22 ± 282.34^{ab}	2398.85 ± 277.92	1855.45 ± 281.90
90	Acetic (n=26,26,28)	2525.06 ± 290.69^{ab}	2969.18 ± 290.65^{abd}	2050.86 ± 281.99
90	Butyric (n=26,22,28)	2855.79 ± 286.45^{ad}	3278.94 ± 288.90^{ad}	2183.19 ± 281.90^{e}
90	Normal (n=30,28,29)	1736.97 ± 274.03^{b}	2274.40 ± 281.78^{b}	2165.41 ± 277.79
90	Propionic (n=26,28,28)	1917.67 ± 282.82^{bde}	2581.48 ± 278.36^{abd}	1877.16 ± 281.90^{e}
105	Acetic (n=26,26,28)	2465.36 ± 290.69^{ab}	2930.68 ± 290.65^{abd}	2089.98 ± 281.99
105	Butyric (n=26,21,28)	3022.22 ± 286.45^{ad}	3137.54 ± 289.59^{ad}	2224.49 ± 281.90^{e}
105	Normal (n=30,28,29)	1701.41 ± 274.03^{b}	2316.55 ± 281.78^{b}	2236.86 ± 277.79
105	Propionic (n=25,26,27)	1984.20 ± 283.32^{bd}	2670.14 ± 279.24^{abe}	1913.72 ± 282.31^{e}
120	Acetic (n=24,26,28)	2555.49 ± 291.68^{ab}	3014.68 ± 290.65^{abd}	2109.28 ± 281.99
120	Butyric (n=26,19,27)	3097.19 ± 286.47^{ad}	3530.10 ± 291.15^{ad}	2269.32 ± 282.33^{e}
120	Normal (n=30,28,29)	1736.84 ± 274.03^{b}	2351.95 ± 281.78^{b}	2318.11 ± 277.79
120	Propionic (n=23,26,27)	2001.86 ± 284.42^{bd}	2715.45 ± 279.26^{abe}	1928.16 ± 282.34^{d}
135	Acetic (n=25,26,28)	2464.58 ± 291.17^{ab}	3211.89 ± 290.65^{ad}	2131.82 ± 281.99
135	Butyric (n=25,18,27)	2933.08 ± 286.95^{ade}	3426.02 ± 292.06^{ad}	2303.60 ± 282.33^{e}
135	Normal (n=30,28,29)	1735.24 ± 274.03^{b}	2382.34 ± 281.78^{b}	2368.90 ± 277.79
135	Propionic (n=23,27,27)	1876.68 ± 284.42^{bd}	2779.99 ± 278.80^{ae}	1938.44 ± 282.34^{d}

SEM=standard error of the mean; Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-5 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
150	Acetic (n=25,26,28)	2489.98 ± 291.17^{ab}	3109.76 ± 290.65^{ad}	2142.26 ± 281.99
150	Butyric (n=24,20,27)	2991.99 ± 287.48^{ade}	3279.97 ± 290.33^{ad}	2346.74 ± 282.33^{e}
150	Normal (n=30,28,28)	1716.46 ± 274.03^{bd}	2415.45 ± 281.78^{be}	2399.15 ± 278.19^{e}
150	Propionic (n=26,26,27)	1871.43 ± 282.82^{bd}	2881.22 ± 279.27^{abe}	1979.12 ± 282.34^{d}
165	Acetic (n=26,26,28)	2471.57 ± 290.69^{ab}	3174.65 ± 290.65^{ad}	2173.57 ± 281.99
165	Butyric (n=23,19,27)	2857.00 ± 288.05^{ade}	3374.03 ± 291.16^{ad}	2372.77 ± 282.33^{e}
165	Normal (n=30,28,29)	1727.71 ± 274.03^{bd}	2415.53 ± 281.78^{be}	2431.53 ± 277.79^{e}
165	Propionic (n=25,27,27)	1848.53 ± 283.32^{bd}	2924.59 ± 278.80^{abe}	2007.59 ± 282.34^{d}
180	Acetic (n=25,26,28)	2440.86 ± 291.17^{ab}	3184.83 ± 290.65^{ad}	2178.39 ± 281.99
180	Butyric (n=25,19,27)	2910.32 ± 286.96^{ade}	3406.97 ± 291.16^{ad}	2405.13 ± 282.33^{e}
180	Normal (n=30,28,29)	1717.73 ± 274.03^{bd}	2463.60 ± 281.78^{be}	2471.85 ± 277.79^{e}
180	Propionic (n=25,27,27)	1866.08 ± 283.31^{bd}	2936.73 ± 278.79^{abe}	2048.19 ± 282.34^{d}
195	Acetic (n=25,24,26)	2481.07 ± 291.16^{ab}	3200.82 ± 291.64^{ad}	2194.50 ± 282.86
195	Butyric (n=22,18,24)	3066.31 ± 288.66^{ade}	3437.48 ± 292.06^{ad}	2432.28 ± 283.79^{e}
195	Normal (n=29,27,28)	1712.50 ± 274.41^{bd}	2487.88 ± 282.20^{be}	2475.94 ± 278.19^{e}
195	Propionic (n=24,26,26)	1787.80 ± 283.83^{bd}	3008.60 ± 279.27^{abe}	2068.19 ± 282.79^{d}
210	Acetic (n=26,26,28)	2548.61 ± 290.69^{b}	3187.05 ± 290.65^{ad}	2285.75 ± 281.99
210	Butyric (n=25,20,27)	3387.61 ± 286.94^{ad}	3128.93 ± 290.32^{ad}	2411.79 ± 282.32^{e}
210	Normal (n=29,28,29)	1864.23 ± 274.41^{b}	2434.94 ± 281.78^{b}	2514.66 ± 277.79
210	Propionic (n=25,25,26)	1923.00 ± 283.29^{bd}	2866.17 ± 279.77^{abe}	2028.98 ± 282.79^{d}

SEM=standard error of the mean; Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 2-5 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	**VFA Solution**	**Mean (mohms/cm^2) ±SEM @ pH 1.5**	**Mean (mohms/cm^2) ±SEM @ pH 4.0**	**Mean (mohms/cm^2) ±SEM @ pH 7.0**
225	Acetic (n=26,26,28)	2895.15 ± 290.69^{a}	3184.88 ± 290.65^{ad}	2137.88 ± 281.99
225	Butyric (n=25,25,28)	3246.44 ± 286.95^{ad}	2879.28 ± 287.12^{abde}	2407.77 ± 281.90^{e}
225	Normal (n=30,28,29)	1975.54 ± 274.03^{b}	2482.24 ± 281.78^{b}	2540.52 ± 277.79
225	Propionic (n=26,25,27)	1916.58 ± 282.79^{bd}	2911.53 ± 279.77^{abe}	2032.94 ± 282.34^{d}
240	Acetic (n=25,26,28)	2911.69 ± 291.16^{a}	3148.69 ± 290.65^{de}	2209.53 ± 281.99
240	Butyric (n=27,25,28)	3248.68 ± 286.01^{ad}	2970.62 ± 287.12^{de}	2436.30 ± 281.90^{e}
240	Normal (n=30,28,29)	2037.73 ± 274.03^{b}	2512.08 ± 281.78	2541.00 ± 277.79
240	Propionic (n=27,25,27)	1980.80 ± 282.33^{bd}	2911.58 ± 279.77^{e}	2064.85 ± 282.34^{d}
255	Acetic (n=24,24,26)	2900.55 ± 291.68^{ah}	2880.08 ± 291.64^{h}	2149.67 ± 282.86^{h}
255	Butyric (n=23,23,26)	3261.33 ± 288.04^{adh}	2988.74 ± 288.25^{deh}	2479.34 ± 282.77^{eh}
255	Normal (n=29,28,28)	2072.74 ± 274.41^{bh}	2539.71 ± 281.78^{h}	2539.93 ± 278.19^{h}
255	Propionic (n=27,24,27)	2083.40 ± 282.33^{bdh}	2894.23 ± 280.29^{eh}	2100.14 ± 282.34^{dh}

SEM=standard error of the mean; Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

There were no significant ($P<0.05$) differences in R values at pH 7.0 (Figure 2-25, Table 2-5).

Acetic acid increased ($P<0.01$) R the greatest at pH 1.5 followed by pH 4.0 and pH 7.0 (Figure 2-26). Butyric acid increased ($P<0.01$) R the greatest at pH 4.0 followed by pH 1.5 and pH 7.0 (Figure 2-27). Normal Ringer's solution increased ($P<0.01$) R at pH 4.0 and 7.0 compared to pH 1.5 (Figure 2-28). Propionic acid increased ($P<0.01$) R at pH 4.0 compared to pH 1.5 and 7.0 (Figure 2-29).

Butyric and acetic acid followed by propionic acid decreased ($P<0.01$) G following the 30 min acclimation period at pH 1.5 and 4.0 (Figure 2-30, 2-31, Table 2-6). There were no significant differences in G at pH 7.0 (Figure 2-32). Acetic acid decreased ($P<0.01$) G at pH 1.5 and 4.0 compared to pH 7.0 (Figure 2-33). Butyric acid decreased ($P<0.01$) G at pH 1.5 compared to pH 4.0 and pH 7.0 (Figure 2-34). Normal Ringer's solution increased ($P<0.01$) G at pH 1.5 compared to pH 4.0 and 7.0 (Figure 2-35). Propionic acid increased ($P<0.01$) G at pH 1.5 and 7.0 compared to pH 4.0 (Figure 2-36).

Homogenized tissues were analyzed by gas chromatography. Significant differences were found only when tissues were exposed to PRS or BRS. When tissues were exposed to PRS, propionic acid absorption was higher ($P<0.01$) compared to exposure to other VFAs or normal Ringer's solution (Table 2-7). When tissues were exposed to butyric acid, butyric acid absorption was higher ($P<0.01$) compared to exposure to other VFAs or normal Ringer's solution (Table 2-8). Butyric acid absorption was also higher ($P<0.01$) at pH 1.5 and 4.0 compared to pH 7.0 (Table 2-9). Butyric acid absorption was highest ($P<0.01$) in tissues exposed to butyric acid at pH 4.0 and butyric acid at pH 1.5 followed by tissues exposed to butyric acid at pH 7.0 (Table 2-10).

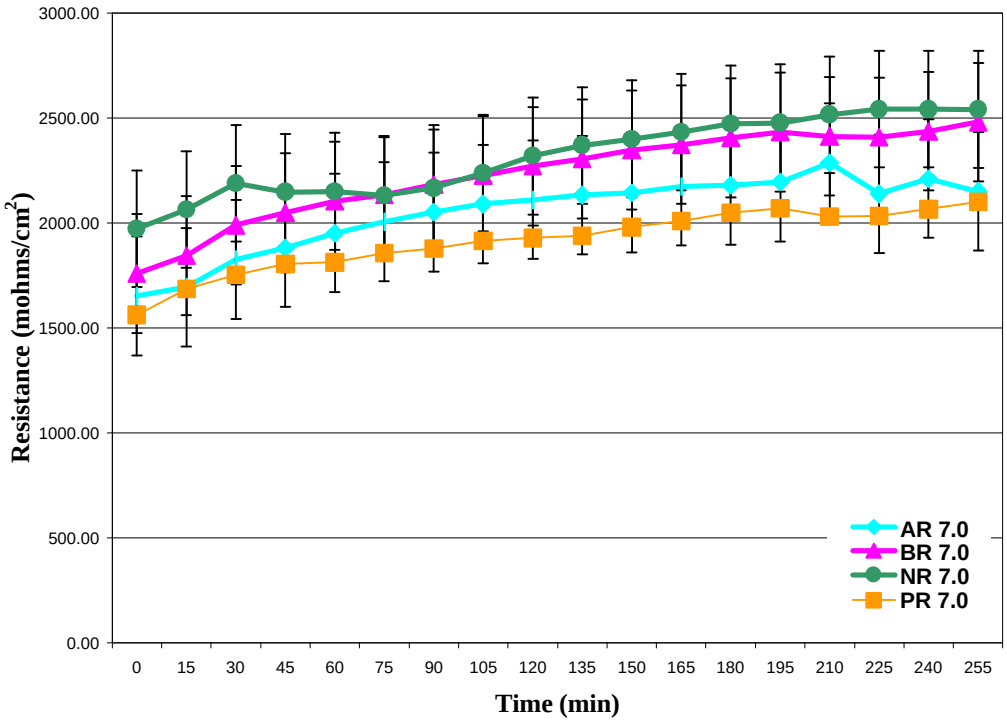


Figure 2-25. Mean Resistance - pH 7.0

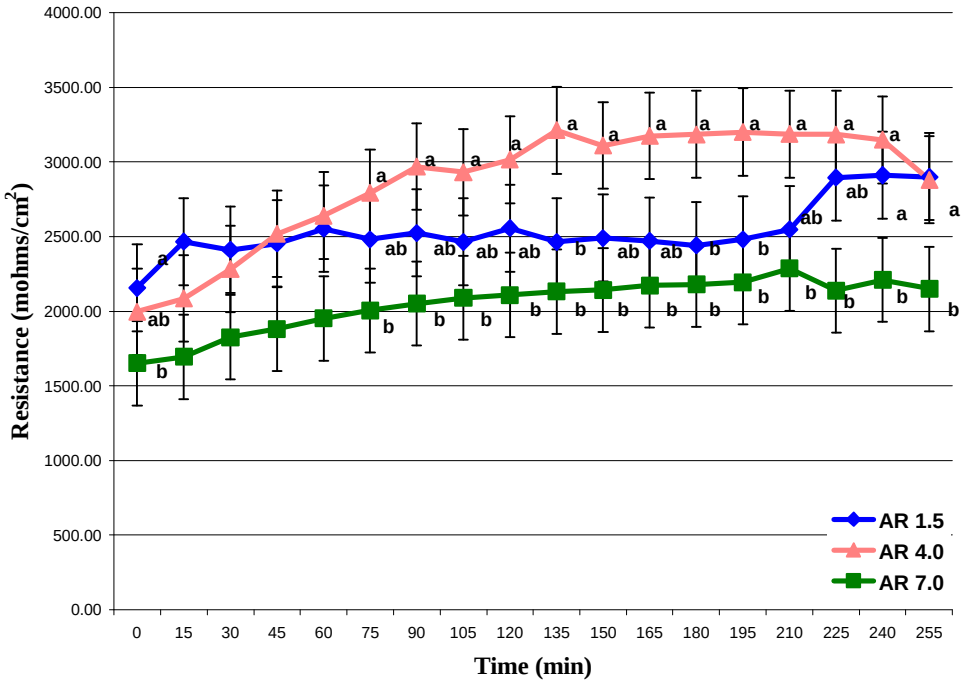


Figure 2-26. Mean Resistance: Acetic Ringer's Solution

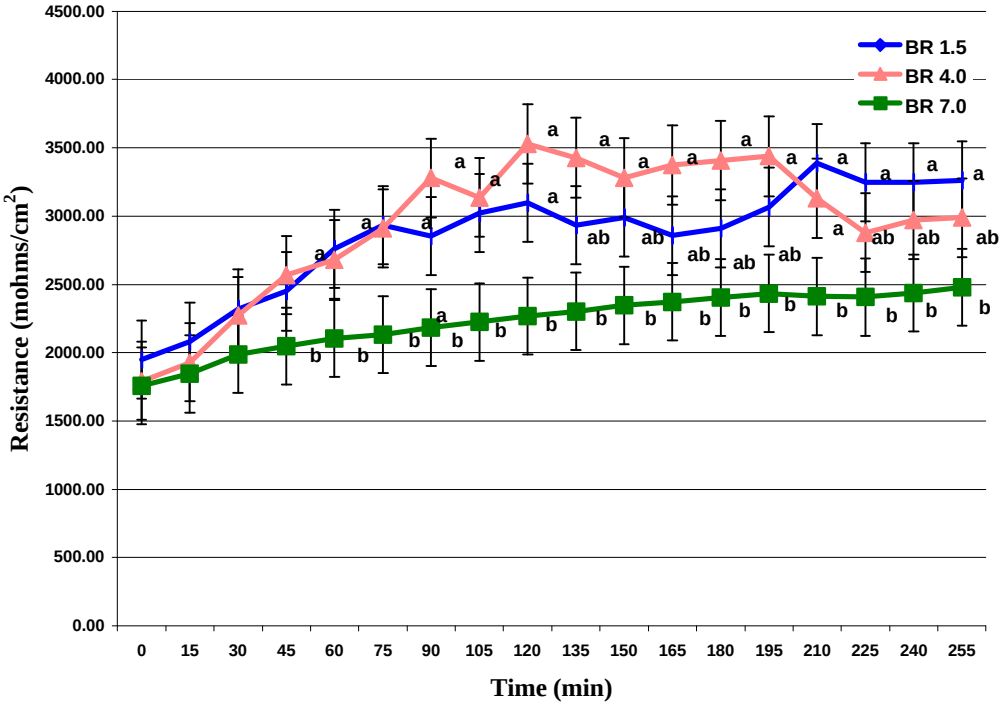


Figure 2-27. Mean Resistance: Butyric Ringer's Solution

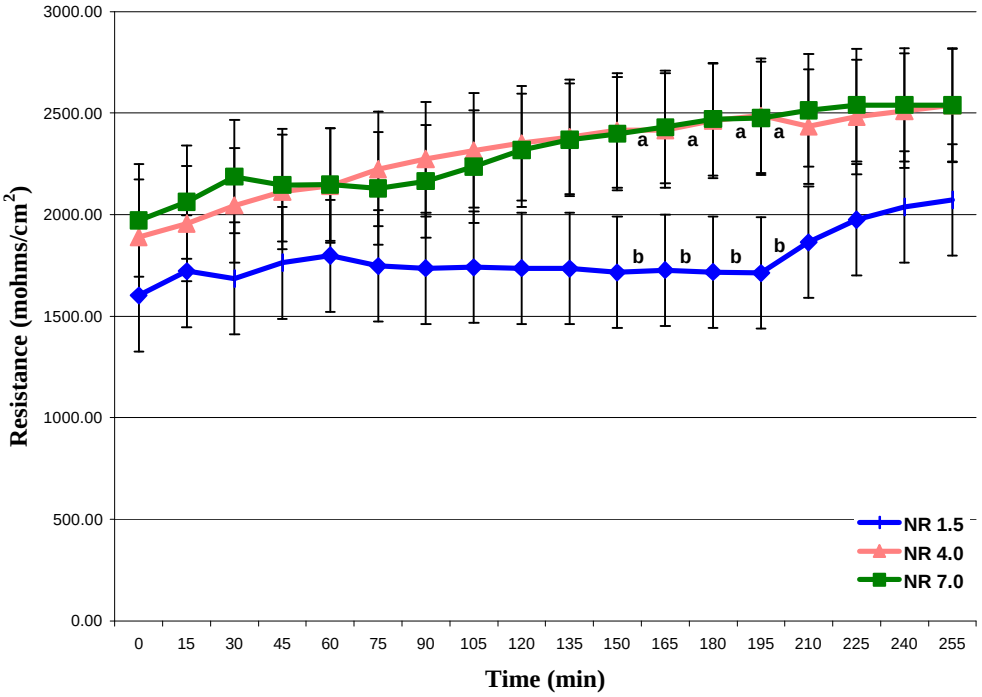


Figure 2-28. Mean Resistance: Normal Ringer's Solution

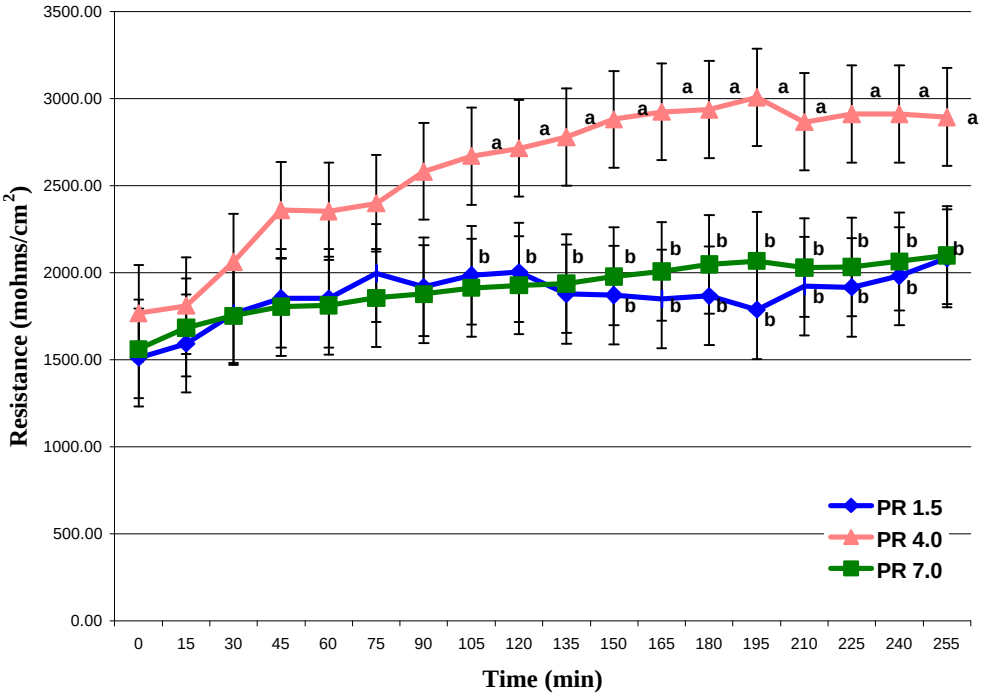


Figure 2-29. Mean Resistance: Propionic Ringer's Solution

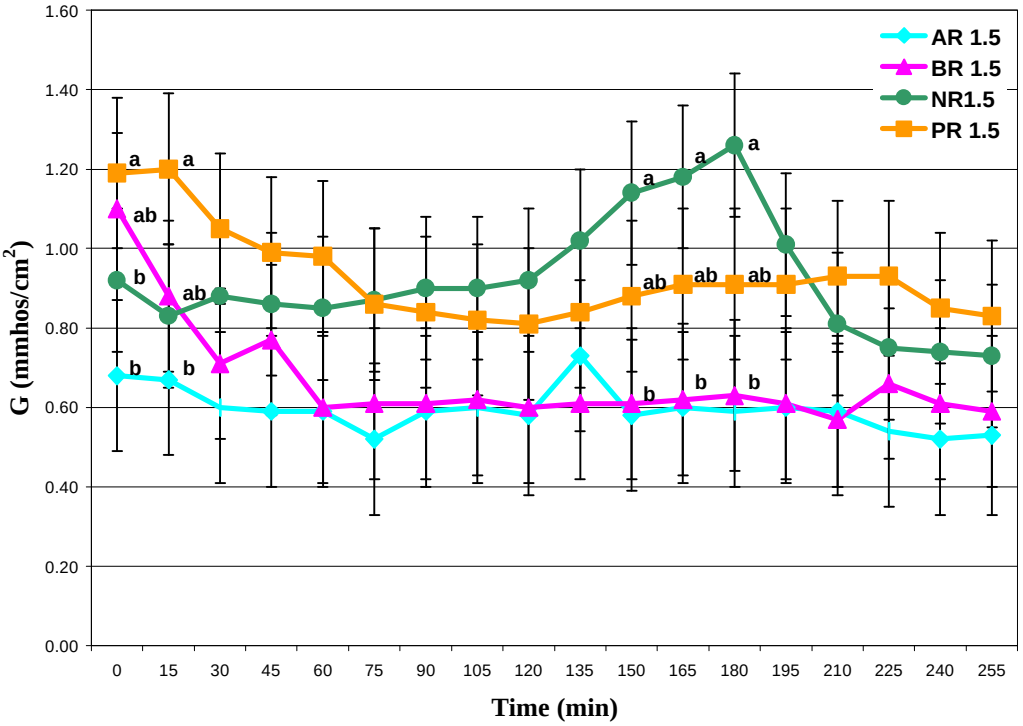


Figure 2-30. Mean Conductance - pH 1.5

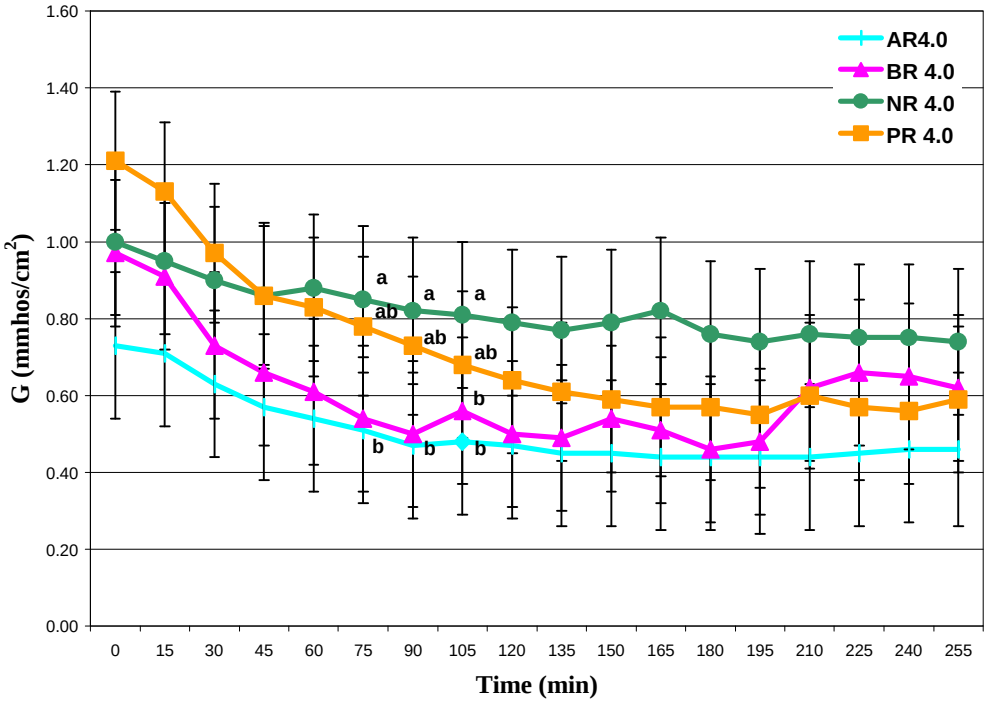


Figure 2-31. Mean Conductance - pH 4.0

Table 2-6. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mmhos/cm^2) ±SEM @ pH 1.5	Mean (mmhos/cm^2) ±SEM @ pH 4.0	Mean (mmhos/cm^2) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	0.68 ± 0.19^{bd}	0.73 ± 0.19^{d}	1.43 ± 0.19^{e}
0	Butyric (n=26,26,27)	1.10 ± 0.19^{ab}	0.97 ± 0.19	1.08 ± 0.19
0	Normal (n=29,26,27)	0.92 ± 0.18^{bd}	1.01 ± 0.19^{d}	1.50 ± 0.19^{e}
0	Propionic (n=28,29,27)	1.19 ± 0.19^{a}	1.21 ± 0.18	1.18 ± 0.19
15	Acetic (n=26,25,28)	0.67 ± 0.19^{bd}	0.71 ± 0.19^{d}	1.28 ± 0.19^{e}
15	Butyric (n=27,27,27)	0.88 ± 0.19^{ab}	0.91 ± 0.19	0.95 ± 0.19
15	Normal (n=28,25,27)	0.83 ± 0.18^{abd}	0.95 ± 0.19^{de}	1.37 ± 0.19^{e}
15	Propionic (n=28,29,28)	1.20 ± 0.19^{a}	1.13 ± 0.18	1.02 ± 0.19
30	Acetic (n=26,25,28)	0.60 ± 0.19^{d}	0.63 ± 0.19^{de}	1.16 ± 0.19^{e}
30	Butyric (n=26,27,27)	0.71 ± 0.19	0.73 ± 0.19	0.90 ± 0.19
30	Normal (n=28,27,28)	0.88 ± 0.18	0.90 ± 0.19	1.33 ± 0.19
30	Propionic (n=27,29,27)	1.05 ± 0.19	0.97 ± 0.18	0.92 ± 0.19
45	Acetic (n=25,25,27)	0.59 ± 0.19^{d}	0.57 ± 0.19^{d}	1.10 ± 0.19^{e}
45	Butyric (n=23,26,27)	0.77 ± 0.19	0.66 ± 0.19	0.88 ± 0.19
45	Normal (n=27,28,29)	0.86 ± 0.18	0.86 ± 0.19	1.28 ± 0.18
45	Propionic (n=27,29,28)	0.99 ± 0.19	0.86 ± 0.18	0.93 ± 0.19
60	Acetic (n=26,26,28)	0.59 ± 0.19^{de}	0.54 ± 0.19^{d}	1.05 ± 0.19^{e}
60	Butyric (n=26,27,28)	0.60 ± 0.19	0.61 ± 0.19	0.86 ± 0.19
60	Normal (n=27,27,27)	0.85 ± 0.18	0.88 ± 0.19	1.25 ± 0.19
60	Propionic (n=27,29,28)	0.86 ± 0.19	0.83 ± 0.18	0.96 ± 0.19
75	Acetic (n=25,26,28)	0.52 ± 0.19^{de}	0.51 ± 0.19^{bd}	1.00 ± 0.19^{e}
75	Butyric (n=26,27,28)	0.61 ± 0.19	0.54 ± 0.19^{b}	0.84 ± 0.19
75	Normal (n=30,28,29)	0.87 ± 0.18	0.85 ± 0.19^{a}	1.18 ± 0.18
75	Propionic (n=27,29,28)	0.84 ± 0.19	0.78 ± 0.18^{ab}	0.89 ± 0.19
90	Acetic (n=26,26,28)	0.59 ± 0.19^{de}	0.47 ± 0.19^{bd}	0.96 ± 0.19^{e}
90	Butyric (n=27,25,28)	0.61 ± 0.19	0.50 ± 0.19^{b}	0.83 ± 0.19
90	Normal (n=30,28,29)	0.90 ± 0.18	0.82 ± 0.19^{a}	1.13 ± 0.18
90	Propionic (n=26,29,28)	0.82 ± 0.19	0.73 ± 0.18^{ab}	0.89 ± 0.19
105	Acetic (n=26,26,28)	0.60 ± 0.19	0.48 ± 0.19^{b}	0.93 ± 0.19
105	Butyric (n=26,23,28)	0.62 ± 0.19	0.56 ± 0.19^{b}	0.81 ± 0.19
105	Normal (n=30,28,29)	0.90 ± 0.18	0.81 ± 0.19^{a}	1.09 ± 0.18
105	Propionic (n=26,27,27)	0.82 ± 0.19	0.68 ± 0.19^{ab}	0.86 ± 0.19

SEM=standard error of the meanMeans not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH

Table 2-6 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mmhos/cm^2) ±SEM @ pH 1.5	Mean (mmhos/cm^2) ±SEM @ pH 4.0	Mean (mmhos/cm^2) ±SEM @ pH 7.0
120	Acetic (n=24,26,28)	0.58 ± 0.20	0.47 ± 0.19	0.91 ± 0.19
120	Butyric (n=26,23,27)	0.60 ± 0.19	0.50 ± 0.19	0.77 ± 0.19
120	Normal (n=30,28,29)	0.92 ± 0.18	0.79 ± 0.19	1.04 ± 0.18
120	Propionic (n=25,28,27)	0.81 ± 0.19	0.64 ± 0.19	0.88 ± 0.19
135	Acetic (n=26,26,28)	0.73 ± 0.19	0.45 ± 0.19	0.88 ± 0.19
135	Butyric (n=25,22,27)	0.61 ± 0.19	0.49 ± 0.19	0.79 ± 0.19
135	Normal (n=30,28,29)	1.02 ± 0.18	0.77 ± 0.19	1.00 ± 0.18
135	Propionic (n=24,29,27)	0.84 ± 0.19	0.61 ± 0.18	0.86 ± 0.19
150	Acetic (n=26,26,28)	0.58 ± 0.19^{b}	0.45 ± 0.19	0.86 ± 0.19
150	Butyric (n=26,23,27)	0.61 ± 0.19^{b}	0.54 ± 0.19	0.77 ± 0.19
150	Normal (n=30,28,28)	1.14 ± 0.18^{a}	0.79 ± 0.19	0.96 ± 0.19
150	Propionic (n=26,28,27)	0.88 ± 0.19^{ab}	0.59 ± 0.19	0.85 ± 0.19
165	Acetic (n=26,26,28)	0.60 ± 0.19^{b}	0.44 ± 0.19	0.84 ± 0.19
165	Butyric (n=25,24,27)	0.62 ± 0.19^{b}	0.51 ± 0.19	0.76 ± 0.19
165	Normal (n=30,28,29)	1.18 ± 0.18^{a}	0.82 ± 0.19	0.92 ± 0.18
165	Propionic (n=26,29,27)	0.91 ± 0.19^{ab}	0.57 ± 0.18	0.84 ± 0.19
180	Acetic (n=26,26,28)	0.59 ± 0.19^{b}	0.44 ± 0.19	0.83 ± 0.19
180	Butyric (n=26,25,27)	0.63 ± 0.19^{b}	0.46 ± 0.19	0.75 ± 0.19
180	Normal (n=30,28,29)	1.26 ± 0.18^{ad}	0.76 ± 0.19^{e}	0.90 ± 0.18^{de}
180	Propionic (n=25,28,27)	0.91 ± 0.19^{ab}	0.57 ± 0.19	0.85 ± 0.19
195	Acetic (n=25,24,26)	0.60 ± 0.19	0.44 ± 0.20	0.80 ± 0.19
195	Butyric (n=22,21,24)	0.61 ± 0.19^{d}	0.48 ± 0.19^{de}	0.74 ± 0.19^{e}
195	Normal (n=29,27,28)	1.01 ± 0.18	0.74 ± 0.19	0.86 ± 0.19
195	Propionic (n=26,28,26)	0.91 ± 0.19	0.55 ± 0.19	0.85 ± 0.19
210	Acetic (n=26,26,28)	0.59 ± 0.19	0.44 ± 0.19	0.83 ± 0.19
210	Butyric (n=26,25,27)	0.57 ± 0.19	0.62 ± 0.19	0.77 ± 0.19
210	Normal (n=30,28,29)	0.81 ± 0.18	0.76 ± 0.19	0.87 ± 0.18
210	Propionic (n=26,27,26)	0.93 ± 0.19	0.60 ± 0.19	0.83 ± 0.19

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH

Table 2-6 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mmhos/cm^2) ±SEM @ pH 1.5	Mean (mmhos/cm^2) ±SEM @ pH 4.0	Mean (mmhos/cm^2) ±SEM @ pH 7.0
225	Acetic (n=26,26,28)	0.54 ± 0.19	0.45 ± 0.19	0.82 ± 0.19
225	Butyric (n=26,26,28)	0.66 ± 0.19	0.66 ± 0.19	0.76 ± 0.19
225	Normal (n=30,28,29)	0.75 ± 0.18	0.75 ± 0.19	0.85 ± 0.18
225	Propionic (n=26,27,27)	0.93 ± 0.19	0.57 ± 0.19	0.79 ± 0.19
240	Acetic (n=25,26,28)	0.52 ± 0.19	0.46 ± 0.19	0.79 ± 0.19
240	Butyric (n=27,26,28)	0.61 ± 0.19	0.65 ± 0.19	0.75 ± 0.19
240	Normal (n=30,28,29)	0.74 ± 0.18^{d}	0.75 ± 0.19^{e}	0.82 ± 0.18^{f}
240	Propionic (n=27,27,27)	0.85 ± 0.19	0.56 ± 0.19	0.78 ± 0.19
255	Acetic (n=24,24,26)	0.53 ± 0.20	0.46 ± 0.20	0.78 ± 0.19
255	Butyric (n=25,24,26)	0.59 ± 0.19	0.62 ± 0.19	0.74 ± 0.19
255	Normal (n=29,28,28)	0.73 ± 0.18	0.74 ± 0.19	0.79 ± 0.19
255	Propionic (n=27,27,27)	0.83 ± 0.19	0.59 ± 0.19	0.76 ± 0.19

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within H

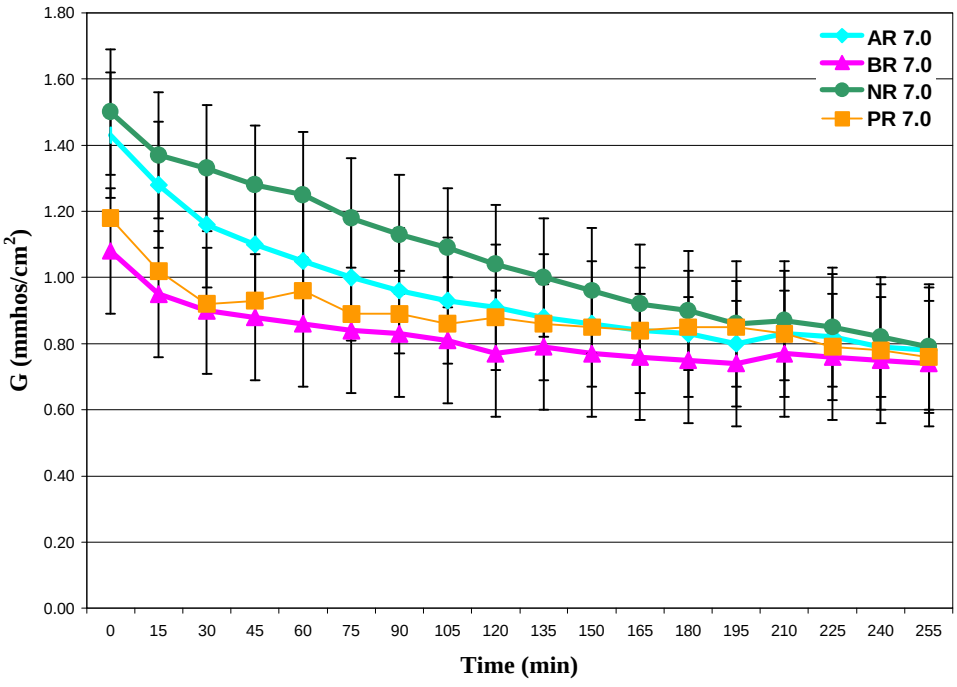


Figure 2-32. Mean Conductance - pH 7.0

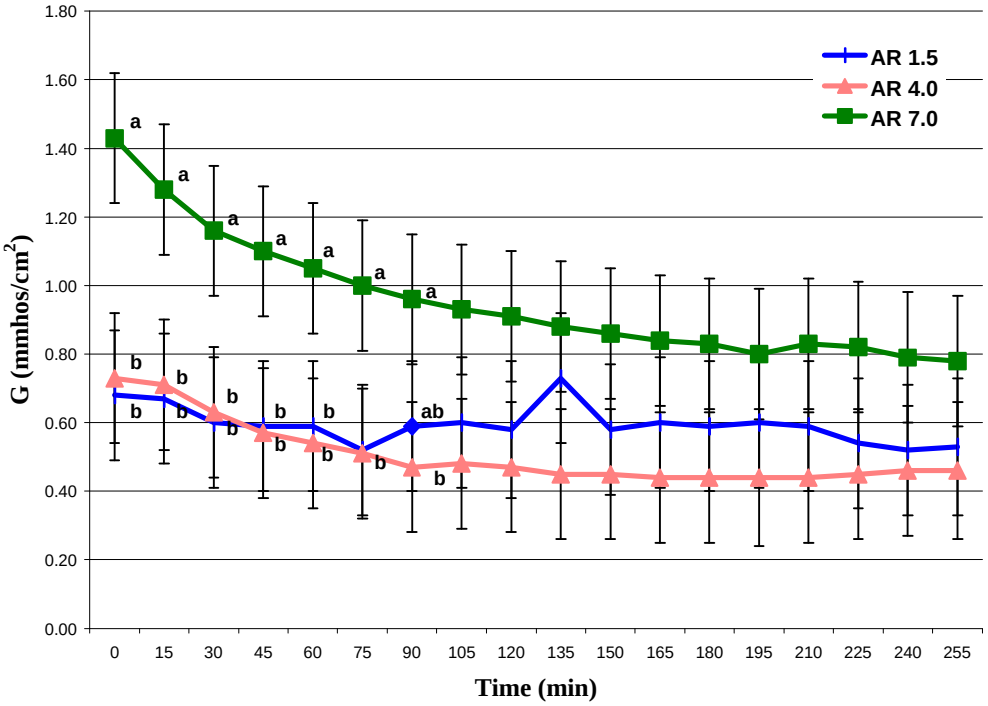


Figure 2-33. Mean Conductance: Acetic Ringer's Solution

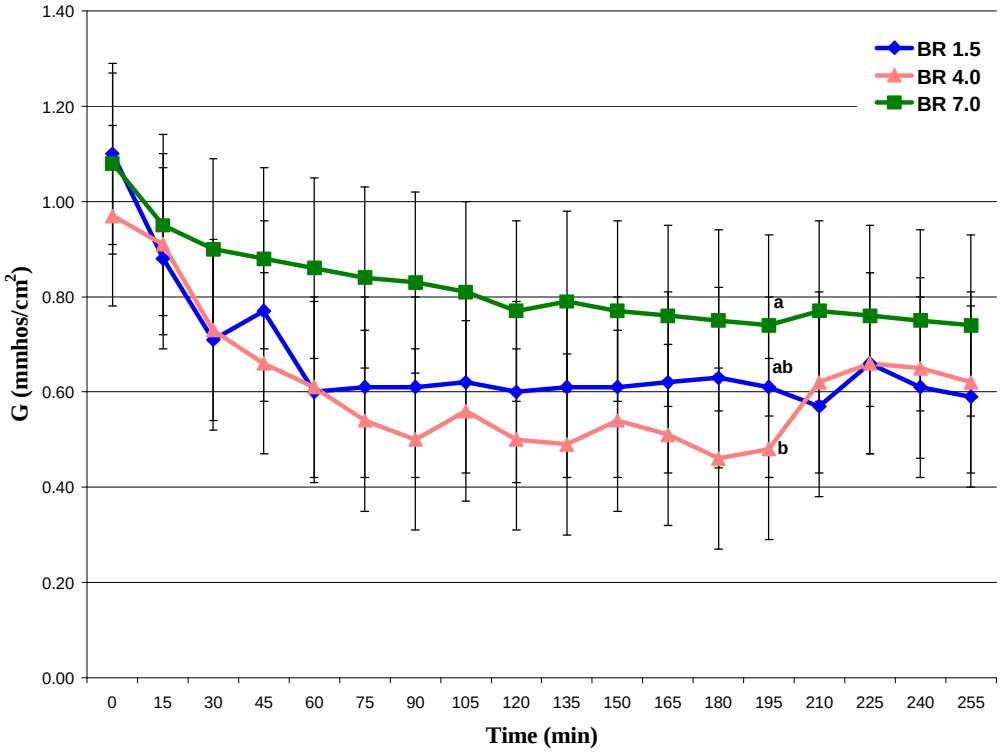


Figure 2-34. Mean Conductance: Butyric Ringer's Solution

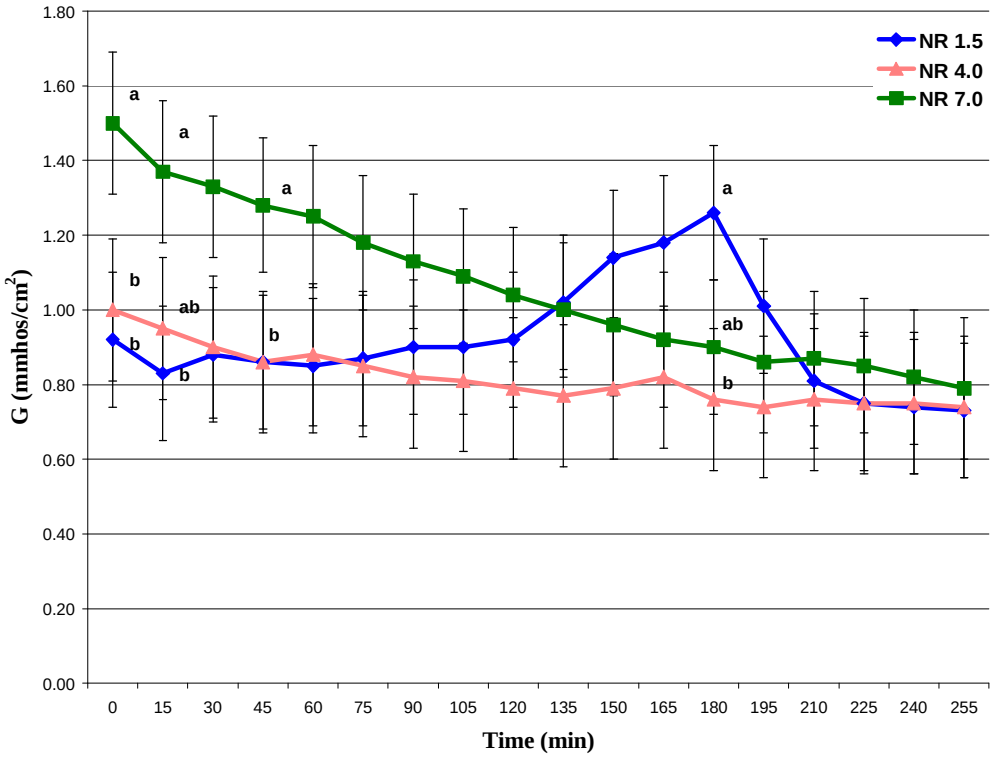


Figure 2-35. Mean Conductance: Normal Ringer's Solution

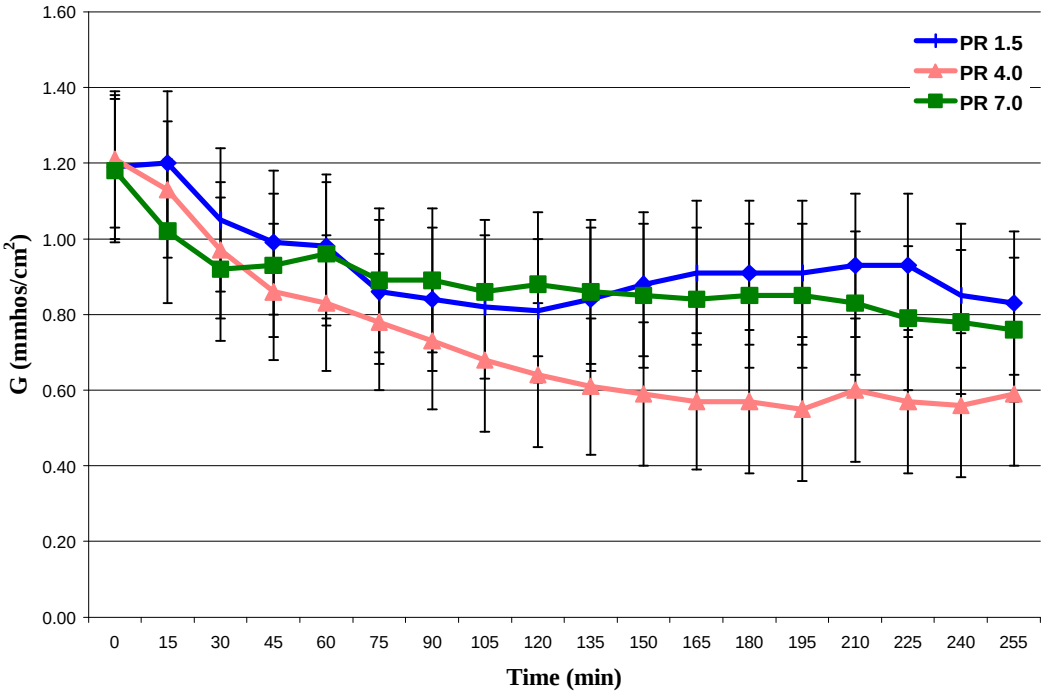


Figure 2-36. Mean Conductance: Propionic Ringer's Solution

Table 2-7. Effect of propionic Ringer's solution exposure on absorption into non-glandular mucosal tissue.

VFA	Mean absorption (mmols/L) ±SEM
Acetic (n=80)	0.16 ± 0.06[a]
Butyric (n=80)	0.27 ± 0.06[a]
Normal (n=82)	0.16 ± 0.06[a]
Propionic (n=84)	0.58 ± 0.06[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 2-8. Concentration of VFAs present after exposure to butyric Ringer's solution into non-glandular mucosal tissue.

VFA	Mean absorption (mmols/L) ±SEM
Acetic (n=80)	0.04 ± 0.06[b]
Butyric (n=80)	0.79 ± 0.06[a]
Normal (n=82)	0.05 ± 0.06[b]
Propionic (n=84)	0.06 ± 0.06[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 2-9. Effect of pH on butyric Ringer's solution exposure on absorption into non-glandular mucosal tissue.

pH	Mean propionic acid concentration (mmols/L) ±SEM
1.5 (n=112)	0.26 ± 0.05[a]
4.0 (n=106)	0.32 ± 0.05[a]
7.0 (n=108)	0.12 ± 0.05[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 2-10. Effect of treatment and pH after exposure on butyric acid absorption into non-glandular mucosal tissue.

VFA and pH	Mean absorption (mmols/L) ±SEM
AR 1.5 (n=27)	0.02 ± 0.10[a]
AR 4.0 (n=26)	0.05 ± 0.10[a]
AR 7.0 (n=27)	0.04 ± 0.10[a]
BR 1.5 (n=28)	0.96 ± 0.10[b]
BR 4.0 (n=27)	1.05 ± 0.10[b]
BR 7.0 (n=25)	0.35 ± 0.10[c]
NR 1.5 (n=29)	0.02 ± 0.09[a]
NR 4.0 (n=25)	0.09 ± 0.10[ac]
NR 7.0 (n=28)	0.03 ± 0.10[a]
PR 1.5 (n=28)	0.03 ± 0.10[a]
PR 4.0 (n=28)	0.10 ± 0.10[ac]
PR 7.0 (n=28)	0.04 ± 0.10[a]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Histopathologic examination of the non-glandular mucosa exposed to normal Ringer's solution at pH 7.0 showed no tissue changes and is shown for reference (Plate A-13). Tissues exposed to pH 1.5 had the highest percent of histologic change compared to tissues exposed to pH 4.0 or pH 7.0 (Table 2-11). Tissues exposed to propionic acid (Table 2-12, Plates A-14 and A-15) or butyric acid (Table 2-12, Plates A-16 and A-17) had the highest percent of histologic change compared to tissues exposed to acetic acid (Table 2-12, Plates A-18 and A-19) or normal Ringer's solution (Table 2-12, Plates A-20 and A-21). The layers most severely affected ($P<0.01$) by histologic change were stratum basale and stratum spinosum combined followed by stratum spinosum, stratum basale, and stratum corneum, respectively (Table 2-13).

Table 2-11. Effect of pH on non-glandular mucosal tissue change.

pH (n=241)	Mean percent of histologic change (%) +SEM
1.5	32.94 ± 2.69[a]
4.0	28.06 ± 2.74[b]
7.0	25.79 ± 3.38[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.05$)

Table 2-12. Effect of treatment on non-glandular mucosal tissue change.

VFA Solution (n=241)	Mean percent of histologic change (%) +SEM
Acetic	18.00 ± 4.43[a]
Butyric	28.51 ± 3.40[b]
Normal	25.79 ± 3.10[ab]
Propionic	30.52 ± 4.02[b]
Valeric	41.83 ± 3.49[c]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 2-13. Differences in depth of penetration of non-glandular mucosal tissue change.

Zone (n=241)	Mean percent of histologic change (%) +SEM
Stratum basale	19.13 ± 4.38[ab]
Stratum corneum	14.25 ± 4.31[b]
Stratum spinosum	26.58 ± 2.28[a]
Stratum basale and spinosum	55.76 ± 4.08[c]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

CHAPTER 5

DISCUSSION

Results of this study suggest that low pH (increased HCl concentration), acetic, butyric and propionic acid induce functional and histologic changes in the non-glandular mucosa of the horse stomach. Also, butyric acid and to a lesser extent, propionic acid, induced functional and histologic damage at pH 4.0. In this study, functional damage was manifested by a decrease in sodium transport that was associated with cell swelling in the layers of the non-glandular mucosa.

Explanation of changes in electrical measurements of the equine non-glandular mucosa is based mainly on studies in other organs and species. The values for short-circuit current and resistance in this study are similar to those found in rabbit esophageal epithelium, porcine non-glandular mucosa, and porcine colon.[1,5,8] This alludes to a similarity of diffusion barriers as well as ion transport mechanisms. Short-circuit current directly measures active ion transport and therefore is a parameter for epithelial function.[1] Previously it has been determined that short-circuit current is exactly equal to the net sodium transport.[9] Previous studies in frog skin,[10] rumen epithelium,[11] and rabbit esophagus[12] found that active sodium absorption accounts for short-circuit current. In rumen epithelium and rabbit esophageal epithelium, it was found that the living layers just below the stratum corneum function in sodium transport, powered by sodium pumps (Na^+K^+ATPases), in cells.[11,12] Recent studies in pig stomach have confirmed that current flow across porcine non-glandular epithelia is due to sodium transport.[13]

In our study, acetic, butyric and propionic acid all decreased ($P<0.01$) sodium transport at pH 1.5. NRS also decreased ($P<0.01$) sodium transport at pH 1.5. Tissues exposed to these VFAs or NRS did not recover to initial values except for acetic acid exposed tissues. Butyric acid exposed tissues had the lowest PCIsc values (-112%). Propionic acid exposed tissues (22%) and acetic acid exposed tissues (35%) also showed significant decreases in PCIsc values. PD was also decreased in BRS and PRS exposed tissues at this pH. The recovery in acetic acid exposed tissues was similar to that seen in porcine non-glandular mucosa.[1] The lack of recovery in tissues exposed to the other VFAs suggests irreversible damage. This loss of function resulted in damage that was observed in tissues examined under light microscopy.

Electrical resistance is a barrier function of the horse stomach with electrical resistance ranging from 2-3000 ohms/cm^2 greater than forestomach epithelium.[11] 30 mmoles/L HCl concentration at pH 1.5 must be present to change R and result in histologic damage to pig and rabbit non-glandular mucosa when applied as long as 3 h to the mucosa *in vitro*.[1,14]

Our results differed from that of Orlando's group in which tissue treated with HCl at pH $\leq$ 1.5 had a slow decrease in tissue R preceding Isc change.[15-17] Isc was affected only when R decreased to $\leq$ 50% of its initial value. In our study, Isc decreased immediately in butyric acid exposed tissues which was then followed by an increase in R. There were no significant changes in G. These results agree with those of Argenzio's group in which tissues exposed to acetate at pH $\leq$ 4.5 underwent a rapid and irreversible abolishment of Isc before any changes in R were detectable.[1] This implies that the non-glandular mucosa is highly permeable to the lipid soluble form of the weak electrolyte

(acetate) and shows that VFAs can penetrate the tissue beneath even without changes in the permeability of the outer barrier. They also proposed a sequence of tissue injury when VFAs are present, which follows. Initially, the undissociated acid penetrates the outer barrier layers and is taken up by living cells beneath the outer layers (Perhaps the VFAs are transported into the cells at the same time as sodium). Intracellular pH is higher in these cells compared to the extracellular fluid, so continued intracellular accumulation and dissociation of the weak acid acidifies the intracellular contents. This disrupts sodium transport (Isc) and cell volume regulation. Therefore, the continuous uptake of water and sodium across the basal membrane cannot be alleviated by basolateral sodium pumping or potassium leakage, resulting in cell swelling and necrosis. These occurrences undermine the outer barrier layers which leads to a secondary decrease in R. They also implied that an H^+ induced decrease in R of the barrier function would enable sodium and water to enter into these underlying layers, hastening the process.

The increase in resistance of the tissues exposed to these VFAs is interesting and different than data reported in the pig. However, an extended unpublished study confirmed that if the tissues were exposed to butyric acid at pH 1.5 for 12 hours a decrease in R and increase in conductance resulted. The increase in resistance seen initially is due to cellular swelling (as seen upon histopathologic examination) causing an increase in intracellular with a concomitant decrease in intercellular space which leads to this initial increase in resistance.[1] This is in contrast to results in the porcine mucosa that showed a decrease in resistance and increase in conductance. Non-glandular tissue in the horse may be more resistant to damage and maintain barrier function longer than porcine mucosa. Tissues exposed to pH 1.5 showed the greatest percent of change (33%)

compared to pH 4 or pH 7 exposed tissues. Propionic (31%) and butyric (29%) exposed tissues also showed the greatest percent of change that correlates well with Isc changes seen functionally. It may be that there is slightly less change due to butyric acid because it may be converted into other metabolites such as B-hydroxybutyrate. Furthermore, tissue VFA concentrations were significantly higher in tissues exposed to the specific VFA. This confirms that VFAs are absorbed in non-glandular tissues of horses.

The histologic change (cellular swelling) that occurred was most severe at pH 1.5 compared to pH 4.0 or pH 7.0. The stratum basale and stratum spinosum layers together were most affected by change (55% of change) compared to either alone or the stratum corneum alone, showing that the VFAs were able to penetrate and cause changes to the deep layers of the tissue.

ATPases have been histochemically located in equine stratified non-glandular mucosa.[18] In the stratum basale, high ATPase activity decreased as superficial layers were neared, showing that there is a gradient of metabolic activity in this mucosa similar to esophageal and rumen epithelium.

Although a 60 mM concentration of acetic, butyric, or propionic acid was not found in the gastric contents of the horses studied, it is possible that the VFAs either volatilized just prior to collection or had already been converted to carbon dioxide, B-hydroxybutyrate, or acetoacetate. Some absorption did occur in the tissues exposed to propionic and butyric acids because tissues exposed to propionic and butyric acids had higher concentrations of these VFAs present at the end of the study compared to other VFAs. A study examined the mucosal uptake and serosal release of VFAs and used the difference between the parameters as an indicator for intraepithelial metabolism or VFA

accumulation.[21] In this study it was found that when propionate (15 mmoles/L) was the only VFA present in mucosal solutions, mean mucosal concentrations decreased to about 13.5 mmoles/L and serosal concentrations increased to 0.8 mmoles/L, resulting in a mean mucosal loss of 18 μmoles/h and a mean serosal gain of 9 μmoles/h. Serosal release was equal to about 50% of mucosal uptake, and the authors felt that this indicated substantial intraepithelial metabolism or accumulation was occurring during the experiment.

The results of this study suggest that butyric acid, propionic acid and acetic acid may cause functional damage to the non-glandular mucosa at pH 1.5. Butyric acid and propionic acid may cause functional damage to the non-glandular mucosa at pH 4.0. Cellular damage induced by acetic acid at an acid pH appears to be reversible as with pig mucosa but the other VFAs produce irreversible damage to the sodium pump that does not recover in 60 minutes. Data presented here suggest that VFAs and HCl damage the sodium transport mechanism in the non-glandular epithelial cell of the equine stomach. This damage results in irreversible changes and eventual ulceration (unpublished data). This suggests that it may be desirable to try to maintain the pH of the horse stomach above 4.0 in order to prevent acid injury. This may be possible by feeding rations that result in a higher pH of the stomach, such as alfalfa hay. Also, diets can be formulated to modify VFA production.

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PART III

PATHOGENESIS OF ACID INJURY IN THE NON-GLANDULAR EQUINE STOMACH: STUDIES WITH ACETIC, BUTYRIC, PROPIONIC, AND VALERIC ACIDS

CHAPTER 1

ABSTRACT

Thirteen horses were necropsied to determine if volatile fatty acids (VFAs) produced from fermentation of carbohydrates (acetic, butyric, propionic, and valeric acids) cause cellular injury which leads to gastric ulceration when exposed to the non-glandular mucosa of the stomach at pH 1.5, 4 and 7. The stomach was removed within one hour of death and dissected along the greater curvature. A 3 cm^2 piece of non-glandular tissue was mounted mucosal side up in an Ussing's chamber. 60 mM of each VFA (acetic, butyric, propionic, or valeric acid) in NRS or NRS alone. NRS was added to the serosal side of the chamber. The pH of the mucosal solution was changed at 30 minutes to pH 1.5, 4 or 7 and exposed for three hours. The solution on the mucosal side was drained and replaced with normal Ringer's solution at pH 7.0 at the end of three hours to determine tissue recovery. Spontaneous potential difference (PD) across tissue and short-circuit current (Isc) (current necessary to nullify PD = sodium transport across tissue) were measured every 15 minutes throughout the study. Percentage of control short-circuit current (PCIsc), resistance (R), and conductance (G) were calculated. Following the Ussing's chambers exposure tissues were placed in 10% formalin and stained with hematoxylin and eosin for routine histopathologic examination. Isc decreased ($P<0.01$) and resistance decreased ($P<0.01$) after 150 min in tissues exposed to NRS at pH 1.5 compared to tissues exposed to NRS at pH 4.0 and 7.0. Isc decreased ($P<0.01$) and resistance increased ($P<0.01$) in tissues exposed to acetic, butyric and propionic acid at an acid pH compared to NRS at all pHs (1.5,4.0,7.0). However, Isc and

tissue resistance both decreased in tissues exposed to valeric acid at all pHs. Also, valeric acid exposed tissues had lower ($P<0.01$) conductance values than acetic acid, butyric acid, normal Ringer's or propionic acid exposed tissues. Also, conductance increased in valeric acid exposed tissues at pH 1.5. Valeric acid (42%), propionic acid (31%) and butyric acid (28%) had the greatest percent ($P<0.01$) of histologic change. NRS (26%) and acetic acid (18%) exposed tissues had the least percent ($P<0.01$) of histologic change. Volatile fatty acids especially valeric acid caused functional and histopathologic changes (cell swelling) at all pHs which could lead to ulceration. The presence of valeric acid in the stomach contents could be the reason that some ulcers continue to form even in an alkaline pH.

CHAPTER 2

INTRODUCTION

The proximal 1/3 of the equine stomach is lined with non-glandular mucosa. Equine gastric ulcer syndrome (EGUS) is common in horses due to the unique anatomical features and the absence of protective barriers found in other species due to the lack of mucus and bicarbonate secretion. Volatile fatty acids have been implicated in the cause of EGUS because at a low pH, they are able to be absorbed by the non-glandular mucosa and found to cause acid injury in other species.

In the pig, 60 mM acetic acid was found to induce reversible acid injury to the non-glandular mucosa.[1] This was determined by measuring the potential difference and short-circuit current across tissue using the *in vitro* Ussing's chamber system. These parameters measure tissue resistance (permeability) and sodium transport. A decrease in resistance and disruption of sodium transport resulted when tissue was damaged by acetic acid. The proposed mechanism for volatile fatty acid injury is related to their high lipid solubility. At a low pH, acetic acid, butyric acid, propionic acid, or valeric acid can become undissociated and easily penetrate the squamous mucosal cell barrier of the stomach. The squamous mucosa lining the proximal stomach in horses may be predisposed to volatile fatty acid injury because of its lack of protective factors (mucus and bicarbonate). Once inside the cell, these weak acids can cause cell acidification, inflammation, and ultimately, ulceration.

VFA concentrations vary in stomach contents of horses. Generally, acetic acid concentrations are the highest in horses fed hay and hay and grain diets.[2] Butyric,

propionic and valeric acid are of less concentration but measurable in horse stomachs.[2,3] The pH of stomach contents containing butyric, propionic and valeric acid has been implicated in EGUS in horses. Generally horses with high stomach concentrations of valeric acid and a low pH in the stomach had more numerous and more severe ulcers than horses with lower concentrations of valeric acid and a high pH in the stomach. However, the effect of VFAs on equine non-glandular mucosa has not been reported. The purpose of the study was to determine effect of pH and VFAs (especially valeric acid) on sodium transport and tissue resistance across the squamous mucosa of the horse stomach in order to elucidate the role of pH and valeric acid in the pathogenesis of gastric ulcer disease in horses.

CHAPTER 3

MATERIALS AND METHODS

Animals and Treatments

Thirteen horses going to necropsy for reasons of debilitation and/or donation were used in this study. Information regarding sex, breed and age were obtained. The horses were humanely euthanatized by lethal injection using an overdose of barbiturate (Beuthanasia®, Schering- Plough, Kenilworth, NJ). Within 1 hour of death, the stomachs from the horses were removed and dissected along the greater curvature to expose the non-glandular mucosa. The stomach contents were removed immediately and placed in two 50 ml vials and frozen at –70° C for later analysis of VFA concentrations. The whole stomach, including non-glandular mucosa**,** was cleaned of food material using deionized water and NRS and examined for gastric ulceration and given a grade based on an equine gastric ulcer scoring system.[4] The non-glandular mucosa was then dissected free from the underlying muscular and serosal tissue and cut into approximately 3.5 cm^2 discs. The tissue was placed in an Ussing's chamber and exposed to valeric acid and other VFAs (acetic, butyric and propionic acids). After VFA exposure, tissue was removed from the chamber and divided in half. One half was placed in 10% formalin for histopathologic examination and the other half was placed in NRS and frozen at –70 C for evaluation of tissue VFA concentration.

Ussing's Chamber Techniques

Gastric tissues were studied in Ussing's chambers based on a previous study by Argenzio and Eisemann.[1] Non-glandular mucosal tissue was pinned mucosal side down in a paraffin tray and bathed in oxygenated normal Ringer's solution maintained at 20 to 22° C (room temperature). The non-glandular mucosa was separated from the underlying muscle layers mounted in Ussing's chambers with an aperture of 7.07 cm^2**.** Tissues were bathed in 10 ml of NRS on the mucosal and serosal surfaces and were oxygenated by circulating the solutions in water-jacket reservoirs with a gas lift bubbled with 95%O_2-5% CO_2. The spontaneous potential difference (PD) was measured by use of Ringer's agar bridges, whose composition was identical to the serosal solution bathing the tissue. The PD was nullified by use of an automatic voltage clamp through Ag-AgCl electrodes. Tissue resistance was calculated from the open circuit PD and from the current necessary to nullify the PD, the short-circuit current (Isc). Calomel electrodes filled with and bathed in saturated KCl were used to record the PD and Isc.

For the study, control tissues were bathed in 10 ml of NRS, with the following composition: in mM: 142 Na, 124 Cl, 25 HCO_3, 10 glucose, 5 K, 1.65 HPO4, 1.25 Ca, 1.1 Mg, and 0.3 H2PO4, on the mucosal and 10 ml of NRS on the serosal surface. For the solutions containing acetic, butyric, propionic, or valeric acids, the sodium salt of the VFA was substituted for an equivalent of sodium chloride so that the final concentration was 60 mM in the NRS resulting in an acetic Ringer's solution (ARS), butyric Ringer's solution (BRS), propionic Ringer's solution (PRS) and valeric Ringer's solution (VRS). The solution containing the VFA (10 ml) was added to the mucosal side of the tissue and 10 ml of NRS was added to the serosal side of the tissue. Since dissimilar solutions were

used in the mucosal and serosal baths, liquid junction potentials (caused by net diffusion of sodium ions from solution to bridge to electrode resulting in PD changes) were present during the electrical measurements. To determine their magnitude and orientation, external circuits were created using the two experimental solutions as described by Orlando et al.[5] Briefly, a KCl bridge connected the chambers. A Ringer's agar bridge connected the calomel electrode to the first solution (NRS). A Ringer's agar bridge also connected the other calomel electrode to the VFA solution. First, the second solution was NRS and the voltage was recorded as the zero voltage. Next, the second solution was changed to the VFA solution and the agar bridge was replaced. Voltage was recorded and this was subtracted from the zero voltage. The result was the junction potential between the two solutions.

The pH (7.0, 4.0, 1.5) of the bathing solution was adjusted by titration with 1 N HCl while measuring with a pH electrode (Accumet A-10 portable pH meter, Fisher Scientific, Pittsburgh, Pa.). For each VFA, chambers were set up with a pH of 1.5, 4, or 7 on the mucosal side for a total of 3 chambers per VFA per experiment. Tissue PD and Isc were measured when each of the three VFAs was studied. Measurements were taken every 15 minutes, and each study lasted for 3.5 hours. The pH of the solution was checked hourly to ensure stability.

At the end of the experiment, conductance, resistance, corrected Isc (corrected for presence of junction potentials), and percent of control Isc were calculated. All of the calculations were based on Ohm's law, V(voltage)=I(current(amps)) x R(resistance(ohms)).

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

Resistance (R): inverse of conductance x 1000

Short-circuit current (Isc) : Isc/ resistance x 1000

Percentage of control Isc: ((cIsc VFA Ringer's solution)/(cIsc normal Ringer's solution)) x 100

VFA Analysis of Tissue and Gastric Contents

To determine uptake of VFAs into the squamous mucosal tissues, the previously frozen one-half disc of tissue was thawed and analyzed by gas chromatography using the following procedure. The tissue was weighed, diced (cut into tiny pieces with a razor) and placed in 5 ml of deionized water. Samples were then homogenized for 5 minutes using the Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Samples were transferred into 15 ml Beckman polypropylene centrifuge tubes and centrifuged at 13,000 rpm for 20 minutes. A 2 ml aliquot was removed from the supernatant and placed into a 5 ml polypropylene test tube. A 1 ml aliquot was removed from the supernatant and placed in a 1.5 ml microcentrifuge tube and cooled on ice. A 0.2 ml internal standard solution (25% phosphoric acid) was added to each aliquot, vortexed at 13,000 rpm for 20 minutes, the supernatant was removed and placed into a gas chromatography vial (~1 ml) and clamped with an aluminum vial closure. The vial was frozen at –70° C for later analysis. Concentrations of VFAs (acetic acid, butyric acid, isobutyric acid, propionic acid, valeric acid, and isovaleric acid) were measured in the homogenized tissue using a gas chromatography method by Playne,[6] modified by Mathew et al[7] (explained in the next section).

Next, the previously frozen gastric contents of 29 of 43 horses (combination of horses used in previous study (30) and present study (13)) were thawed, and approximately 4 g of the gastric contents were centrifuged at 15,000 x g at 4° C for 15 min in a Beckman Model J-21C Centrifuge with a JA-20 rotor (Beckman Instruments, Palo Alto, CA). One and one-half milliliters of supernatant were added to 300 µl of 25% metaphosphoric acid made from ortho-phosphoric acid (Fisher Scientific Company, Pittsburgh, PA) at a 5:1 ratio and incubated at room temperature for 30 min. After centrifugation to remove the precipitate, 1 µl of sample (either of the homogenized tissue preparation described above or the gastric contents) was injected into a Hewlett Packard Model 5890 Gas Chromatograph (Hewlett Packard, Avondale, PA) with an HP-FFAP 10 m x 0.53 mm x 1 µm capillary column packed with cross-linked polyethylene glycol-TPA. A flame ionization detector (Hewlett Packard, Avondale, PA) was used with an injector temperature of 200° C, an oven temperature of 80° C, and a detector temperature of 250° C. The carrier gas was helium with a flow rate of 30 ml/min. The linear velocity was 26 cm/sec. Head pressure was 1 psi. The standards used were acetic acid, butyric acid, isobutyric acid, propionic acid, valeric acid and isovaleric acid (Alltech Associates, Inc. Deerfield, IL) and an internal standard of 2-ethylbutyric acid (Eastman Kodak Inc., Rochester, NY). The retention times were approximately 8.38 min for acetic acid, 12.68 min for butyric acid, 11.49 min for isobutyric acid, 10.88 min for propionic acid, 15.54 min for valeric acid, 13.60 min for isovaleric acid, and 16.19 min for 2-ethylbutyric acid.

Preparation for Histopathologic Examination

The other half of the tissues placed in 10% formalin, was then prepared for histologic examination. Tissues were trimmed and embedded in paraffin, sectioned at 5 μm using a microtome, placed on glass slides, and stained with hematoxylin and eosin for examination under light microscopy. Slides were examined under light microscopy to determine amount of cell swelling or necrosis present. Measurements were taken using the Bio Quant (R & M Biometrics Inc., Nashville, TN) computer program (True Color Windows Advanced Image Analysis System). A thickness measurement was taken from the luminal surface of the keratinized layer (stratum corneum) to the beginning of the lamina propria to determine depth of tissue (L1). Five measurements were taken along the same region of uniform tissue and a mean was calculated for each of the 2 measurements. To calculate percent cell swelling, a thickness measurement was taken across the layer of cell swelling (L2) when present. Percent cell swelling was calculated using the formula L2/L1 x 100. The location of cell swelling (stratum corneum, stratum spinosum, or stratum basale or a combination) was also recorded.

Statistical Analyses

Data were analyzed using the mixed procedure of SAS. The model used for each tissue type and variable was a completely randomized design split-plot ANOVA with replicates, with treatment (solution conditions) in the main plot and time and time X treatment interaction in the subplot. Time was a repeated measures factor. Least squares means and pooled standard errors of the mean were calculated and a least significant difference mean separation was performed. $P < 0.05$ was considered significant.

CHAPTER 4

RESULTS

Tissues obtained from the horses used in the Ussing's chamber system were grossly normal. Information regarding age (Table A-7), breed (Table A-8), sex (Table A-9), reason for euthanasia (Table A-10), *Gastrophilus intestinalis* larvae (bots) present in the stomach (Table A-11) and ulceration present (Table A-12) was obtained. None of these characteristics was found to be a statistically significant influence on the parameters studied.

In the gastric contents collected from each stomach within 1 hour acetic acid was highest in concentration followed by isobutyric, propionic, butyric, isovaleric and valeric acid, respectively (Table 3-1).

Table 3-1. Mean volatile fatty acid concentration of gastric contents obtained from 29 of the necropsied horses.

VFA Solution	**Mean (mmols/L) ±SEM**
Acetic	16.23 ± 2.78
Propionic	0.67 ± 0.28
Butyric	0.30 ± 0.08
Isobutyric	1.09 ± 0.17
Valeric	0.02 ± 0.01
Isovaleric	0.09 ± 0.17

Valeric acid decreased ($P<0.01$) Isc following the 30 min acclimation period at pH 1.5. Isc remained low throughout the experimental period and never reached levels of tissues exposed to other VFAs or NRS during the last hour when valeric acid was removed and replaced with NRS. Acetic acid, butyric acid, propionic acid and valeric acid decreased ($P<0.01$) Isc following the 30 min acclimation period at pH 1.5 (Figure 3-1, 3-2, Table 3-2).

Valeric acid decreased ($P<0.01$) Isc following the 30 min acclimation period at pH 4.0. Butyric acid and propionic acid decreased ($P<0.01$) Isc following the 30 min acclimation period at pH 4.0. In all but valeric acid exposed tissues, there was some, but not complete recovery following 195 min of exposure (Figure 3-3 and 3-4, Table 3-2).

Valeric acid decreased ($P<0.01$) Isc following the 30 min acclimation period at pH 4.0. In all but valeric acid exposed tissues, there was some, but not complete recovery following 195 min of exposure (Figure 3-5, Table 3-2).

Acetic, butyric, and propionic acid decreased ($P<0.01$) Isc at pH 1.5 and 4.0 compared to pH 7.0 (Figure 3-6, 3-7, 3-8). There was a trend for normal Ringer's to decrease Isc at pH 1.5 and 4.0 compared to pH 7.0 (Figure 3-9). Valeric acid decreased ($P<0.01$) Isc at pH 1.5 compared to pH 7.0 (Figure 3-10).

Valeric acid decreased ($P<0.01$) PCIsc following the 30 min acclimation period at pH 1.5 (Figure 3-11, Table 3-3). There were no significant differences in PCIsc at pH 4.0 (Figure 3-12, Table 3-3). Valeric acid decreased ($P<0.01$) PCIsc following the 30 min acclimation period at pH 7.0 (Figure 3-13, Table 3-3).

Acetic acid and propionic acid decreased ($P<0.01$) PCIsc at pH 1.5 followed by pH 4.0 compared to pH 7.0 (Figure 3-14, 3-15). Butyric acid decreased ($P<0.01$) PCIsc

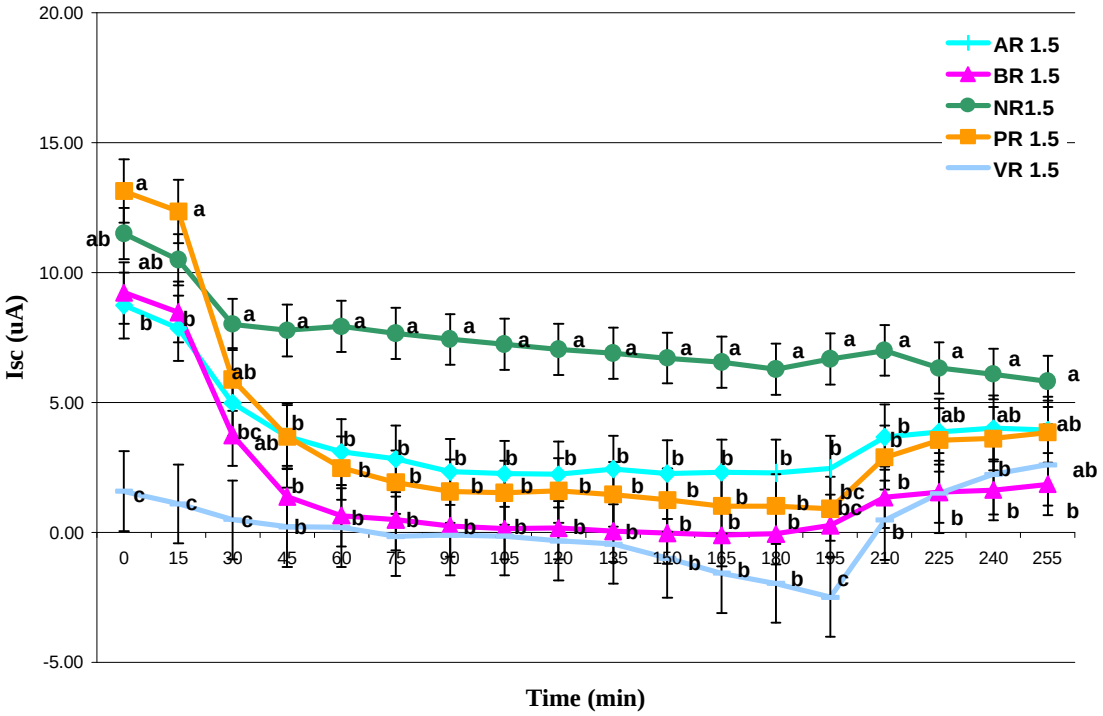


Figure 3-1. Mean Short-Circuit Current (Comparisons with VR) - pH 1.5

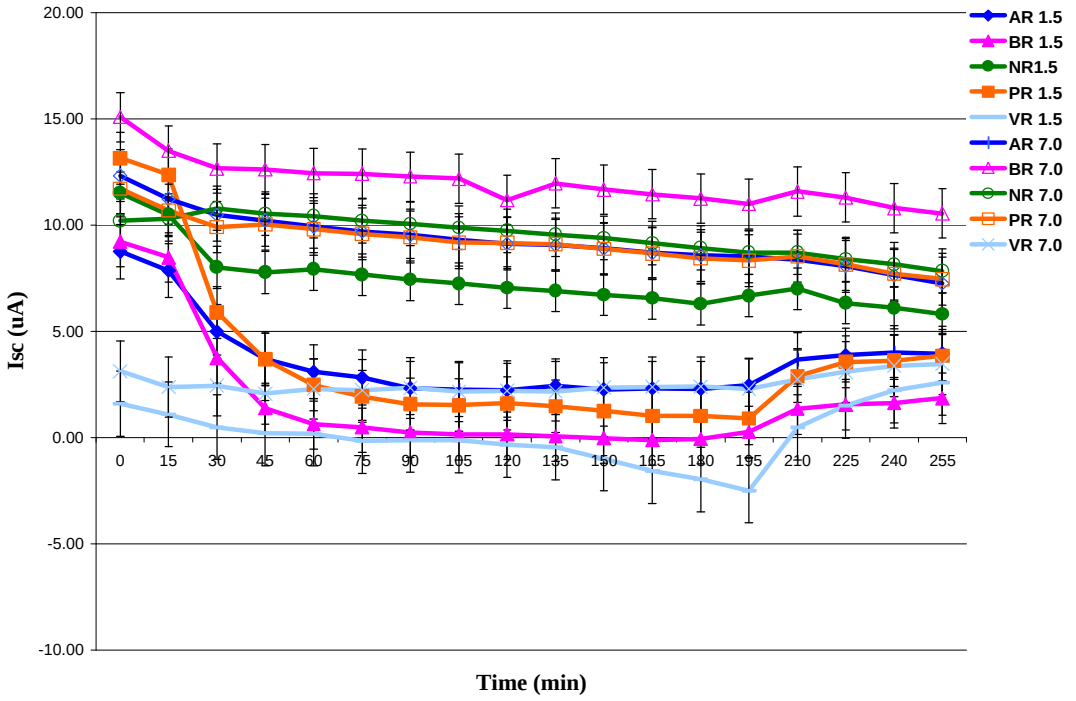


Figure 3-2. Mean Short-Circuit Current (Comparisons with VR) - pH 1.5 & 7.0

Table 3-2. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (μA) ±SEM @ pH 1.5	Mean (μA) ±SEM @ pH 4.0	Mean (μA) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	8.74 ± 1.27^{bdg}	11.78 ± 1.27^{adeg}	12.32 ± 1.22^{abeg}
0	Butyric (n=29,29,29)	9.22 ± 1.18^{bdg}	11.97 ± 1.16^{dg}	15.08 ± 1.16^{aeg}
0	Normal (n=40,34,37)	11.51 ± 0.99^{abg}	9.29 ± 1.08^{ag}	10.19 ± 1.04^{bg}
0	Propionic (n=28,29,27)	13.14 ± 1.22^{adg}	9.35 ± 1.19^{aeg}	11.71 ± 1.22^{bdeg}
0	Valeric (n=16,17,18)	1.58 ± 1.53^{cg}	1.80 ± 1.47^{bg}	3.11 ± 1.43^{c}
15	Acetic (n=26,25,28)	7.86 ± 1.26^{ab}	10.95 ± 1.27^{a}	11.24 ± 1.22 ^{abe}
15	Butyric (n=30,31,29)	8.47 ± 1.17^{bd}	11.50 ± 1.16^{ade}	13.49 ± 1.16^{ae}
15	Normal (n=39,31,36)	10.49 ± 0.99^{ab}	9.38 ± 1.09^{a}	10.29 ± 1.04^{b}
15	Propionic (n=28,29,28)	12.36 ± 1.22^{ad}	8.89 ± 1.19^{ae}	10.68 ± 1.22^{abde}
15	Valeric (n=17,18,17)	1.09 ± 1.52^{c}	1.66 ± 1.45^{b}	2.37 ± 1.43^{c}
30	Acetic (n=26,25,28)	4.98 ± 1.26^{abd}	6.58 ± 1.27^{ad}	10.47 ± 1.22^{ae}
30	Butyric (n=29,31,30)	3.74 ± 1.18^{bcd}	5.69 ± 1.16^{ad}	12.66 ± 1.16^{ae}
30	Normal (n=38,35,37)	8.01 ± 0.99^{ade}	7.72 ± 1.08^{ae}	10.78 ± 1.04^{ad}
30	Propionic (n=27,29,27)	5.88 ± 1.22^{abd}	5.74 ± 1.19^{ad}	9.90 ± 1.22^{ae}
30	Valeric (n=17,16,17)	0.48 ± 1.52^{c}	1.57 ± 1.48^{b}	2.44 ± 1.43^{b}
45	Acetic (n=25,25,27)	3.69 ± 1.27^{bd}	6.30 ± 1.27^{abd}	10.21 ± 1.22^{ae}
45	Butyric (n=26,30,30)	1.37 ± 1.19^{bd}	3.39 ± 1.16^{bcd}	12.61 ± 1.16^{ae}
45	Normal (n=40,34,38)	7.77 ± 0.99^{a}	8.05 ± 1.08^{a}	10.53 ± 1.03^{a}
45	Propionic (n=27,29,28)	3.68 ± 1.22^{bd}	4.70 ± 1.19^{bcd}	10.03 ± 1.22^{ae}
45	Valeric (n=16,18,16)	0.20 ± 1.53^{b}	1.50 ± 1.46^{c}	2.05 ± 1.44^{b}
60	Acetic (n=26,26,28)	3.09 ± 1.26^{bd}	5.88 ± 1.26^{abd}	9.92 ± 1.22^{ae}
60	Butyric (n=29,29,31)	0.64 ± 1.18^{bd}	2.50 ± 1.16^{bcd}	12.44 ± 1.16^{ae}
60	Normal (n=40,34,38)	7.92 ± 0.99^{a}	8.17 ± 1.08^{a}	10.42 ± 1.03^{a}
60	Propionic (n=26,29,28)	2.46 ± 1.22^{bd}	3.81 ± 1.19^{bcd}	9.80 ± 1.22^{ae}
60	Valeric (n=17,17,17)	0.18 ± 1.52^{b}	1.23 ± 1.47^{c}	2.28 ± 1.43^{b}
75	Acetic (n=23,26,28)	2.82 ± 1.28^{bd}	5.38 ± 1.26^{abd}	9.69 ± 1.22^{ae}
75	Butyric (n=29,29,31)	0.48 ± 1.18^{bd}	2.13 ± 1.16^{bcd}	12.41 ± 1.16^{ae}
75	Normal (n=43,35,39)	7.66 ± 0.98^{a}	8.05 ± 1.08^{a}	10.19 ± 1.03^{a}
75	Propionic (n=27,29,28)	1.92 ± 1.22^{bd}	3.28 ± 1.19^{bcd}	9.58 ± 1.22^{ae}
75	Valeric (n=16,17,17)	-0.16 ± 1.53^{b}	1.17 ± 1.47^{c}	2.23 ± 1.43^{b}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-2 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (μA) ±SEM @ pH 1.5	Mean (μA) ±SEM @ pH 4.0	Mean (μA) ±SEM @ pH 7.0
90	Acetic (n=26,26,28)	2.32 ± 1.26^{bd}	5.05 ± 1.26^{abde}	9.53 ± 1.22^{ae}
90	Butyric (n=29,26,31)	0.24 ± 1.18^{bd}	1.62 ± 1.18^{bcd}	12.28 ± 1.16^{ae}
90	Normal (n=43,36,39)	7.43 ± 0.98^{a}	7.88 ± 1.07^{a}	10.05 ± 1.03^{a}
90	Propionic (n=26,28,28)	1.56 ± 1.22^{bd}	2.78 ± 1.20^{bcd}	9.42 ± 1.22^{ae}
90	Valeric (n=16,14,18)	-0.12 ± 1.53^{b}	1.18 ± 1.50^{c}	2.33 ± 1.43^{b}
105	Acetic (n=26,26,28)	2.25 ± 1.26^{bd}	4.86 ± 1.26^{abd}	9.30 ± 1.22^{ae}
105	Butyric (n=29,25,31)	0.13 ± 1.18^{bd}	1.49 ± 1.18^{bd}	12.18 ± 1.28^{ae}
105	Normal (n=43,36,39)	7.24 ± 0.98^{a}	7.66 ± 1.07^{a}	10.05 ± 1.03^{a}
105	Propionic (n=25,26,27)	1.51 ± 1.23^{bd}	2.53 ± 1.21^{bd}	9.17 ± 1.22^{ae}
105	Valeric (n=17,15,19)	-0.14 ± 1.53^{b}	1.23 ± 1.49^{b}	2.16 ± 1.42^{b}
120	Acetic (n=24,26,28)	2.22 ± 1.27^{bd}	4.73 ± 1.26^{abd}	9.12 ± 1.22^{ae}
120	Butyric (n=29,23,30)	0.15 ± 1.18^{bd}	1.47 ± 1.19^{bd}	11.17 ± 1.16^{ae}
120	Normal (n=43,36,39)	7.04 ± 0.98^{ad}	7.32 ± 1.07^{ade}	9.73 ± 1.03^{ae}
120	Propionic (n=23,26,27)	1.59 ± 1.24^{bd}	2.34 ± 1.21^{bd}	9.16 ± 1.22^{ae}
120	Valeric (n=16,14,21)	-0.33 ± 1.53^{b}	1.11 ± 1.50^{b}	2.20 ± 1.40^{b}
135	Acetic (n=25,26,28)	2.44 ± 1.27^{bd}	4.64 ± 1.26^{abd}	9.04 ± 1.22^{ae}
135	Butyric (n=28,22,30)	0.05 ± 1.18^{bd}	1.38 ± 1.20^{bd}	11.96 ± 1.16^{ae}
135	Normal (n=43,36,39)	6.90 ± 0.98^{ad}	7.20 ± 1.07^{ade}	9.55 ± 1.03^{ae}
135	Propionic (n=23,27,27)	1.46 ± 1.24^{bd}	2.16 ± 1.20^{bd}	9.10 ± 1.22^{ae}
135	Valeric (n=16,15,21)	-0.46 ± 1.53^{b}	1.02 ± 1.49^{b}	2.16 ± 1.40^{b}
150	Acetic (n=25,26,28)	2.26 ± 1.27^{bd}	4.58 ± 1.26^{abd}	8.90 ± 1.22^{ae}
150	Butyric (n=27,24,30)	-0.03 ± 1.19^{bd}	1.18 ± 1.19^{bd}	11.67 ± 1.16^{ae}
150	Normal (n=43,36,38)	6.71 ± 0.98^{ad}	7.19 ± 1.07^{ade}	9.39 ± 1.03^{ae}
150	Propionic (n=26,26,27)	1.24 ± 1.22^{bd}	2.09 ± 1.21^{bd}	8.87 ± 1.22^{ae}
150	Valeric (n=17,15,21)	-1.00 ± 1.52^{b}	1.17 ± 1.49^{b}	2.35 ± 1.40^{b}
165	Acetic (n=26,26,28)	2.31 ± 1.26^{bd}	4.51 ± 1.26^{abd}	8.68 ± 1.22^{ae}
165	Butyric (n=26,23,30)	-0.12 ± 1.19^{bd}	1.18 ± 1.19^{bd}	11.44 ± 1.16^{ae}
165	Normal (n=43,36,39)	6.55 ± 0.98^{ad}	6.78 ± 1.07^{ade}	9.14 ± 1.03^{ae}
165	Propionic (n=25,27,27)	1.01 ± 1.23^{bd}	1.84 ± 1.20^{bd}	8.65 ± 1.22^{ae}
165	Valeric (n=17,15,21)	-1.58 ± 1.52^{b}	1.08 ± 1.49^{b}	2.38 ± 1.40^{b}
180	Acetic (n=25,26,28)	2.29 ± 1.27^{bd}	4.51 ± 1.26^{abd}	8.57 ± 1.22^{ae}
180	Butyric (n=28,23,30)	-0.07 ± 1.18^{bd}	1.15 ± 1.19^{bd}	11.25 ± 1.16^{ae}
180	Normal (n=43,36,39)	6.28 ± 0.98^{ad}	6.76 ± 1.07^{ade}	8.92 ± 1.03^{a}
180	Propionic (n=25,27,27)	1.01 ± 1.23^{bd}	1.91 ± 1.20^{bd}	8.44 ± 1.22^{ae}
180	Valeric (n=17,15,21)	-1.97 ± 1.52^{bd}	1.07 ± 1.49^{bde}	2.39 ± 1.40^{be}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-2 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (μA) ±SEM @ pH 1.5	Mean (μA) ±SEM @ pH 4.0	Mean (μA) ±SEM @ pH 7.0
195	Acetic (n=25,24,26)	2.45 ± 1.27^{bd}	4.68 ± 1.27^{abd}	8.51 ± 1.22^{ae}
195	Butyric (n=24,22,27)	0.26 ± 1.20^{bcd}	1.32 ± 1.20^{bd}	10.99 ± 1.17^{ae}
195	Normal (n=41,35,38)	6.67 ± 0.99^{a}	6.54 ± 1.08^{ade}	8.71 ± 1.03^{a}
195	Propionic (n=24,26,26)	0.89 ± 1.23^{bd}	1.76 ± 1.21^{bd}	8.32 ± 1.22^{ae}
195	Valeric (n=17,16,21)	-2.50 ± 1.52^{cd}	1.02 ± 1.48^{bde}	2.29 ± 1.40^{be}
210	Acetic (n=26,26,28)	3.67 ± 1.26^{bd}	4.17 ± 1.26^{abd}	8.36 ± 1.22^{ae}
210	Butyric (n=27,24,30)	1.34 ± 1.19^{bd}	1.71 ± 1.19^{bd}	11.58 ± 1.16^{ae}
210	Normal (n=42,35,39)	7.00 ± 0.98^{a}	6.10 ± 1.08^{a}	8.71 ± 1.03^{a}
210	Propionic (n=24,25,26)	2.87 ± 1.23^{bd}	2.04 ± 1.21^{bd}	8.53 ± 1.22^{ae}
210	Valeric (n=16,13,16)	0.47 ± 1.53^{b}	1.30 ± 1.51^{b}	2.74 ± 1.44^{b}
225	Acetic (n=26,26,28)	3.87 ± 1.26^{abd}	4.67 ± 1.26^{abd}	8.06 ± 1.22^{ae}
225	Butyric (n=27,29,31)	1.55 ± 1.19^{bd}	2.22 ± 1.16^{bd}	11.29 ± 1.16^{ae}
225	Normal (n=43,35,38)	6.32 ± 0.98^{a}	6.19 ± 1.08^{a}	8.38 ± 1.03^{a}
225	Propionic (n=26,25,27)	3.55 ± 1.22^{abd}	2.67 ± 1.21^{bd}	8.14 ± 1.22^{ae}
225	Valeric (n=15,16,17)	1.50 ± 1.54^{b}	1.47 ± 1.48^{b}	3.09 ± 1.43^{b}
240	Acetic (n=25,26,28)	4.00 ± 1.27^{abd}	5.18 ± 1.26^{abde}	7.63 ± 1.22^{ae}
240	Butyric (n=29,29,31)	1.63 ± 1.18^{bd}	2.35 ± 1.16^{bd}	10.80 ± 1.16^{ae}
240	Normal (n=43,35,38)	6.09 ± 0.98^{a}	6.16 ± 1.08^{a}	8.14 ± 1.03^{a}
240	Propionic (n=27,25,27)	3.61 ± 1.22^{abd}	3.16 ± 1.21^{bd}	7.70 ± 1.22^{ae}
240	Valeric (n=16,14,16)	2.22 ± 1.53^{b}	1.65 ± 1.50^{b}	3.37 ± 1.44^{b}
255	Acetic (n=24,24,26)	3.95 ± 1.27^{abh}	5.43 ± 1.27^{ah}	7.25 ± 1.22^{ah}
255	Butyric (n=26,27,29)	1.85 ± 1.19^{bdh}	2.33 ± 1.17^{bdh}	10.54 ± 1.16^{aeh}
255	Normal (n=42,28,37)	5.81 ± 0.98^{ah}	5.86 ± 1.08^{ah}	7.83 ± 1.04^{ah}
255	Propionic (n=27,24,27)	3.85 ± 1.22^{abdh}	3.34 ± 1.22^{abdh}	7.45 ± 1.22^{aeh}
255	Valeric (n=14,14,16)	2.59 ± 1.56^{bg}	1.72 ± 1.50^{bg}	3.46 ± 1.44^{bg}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

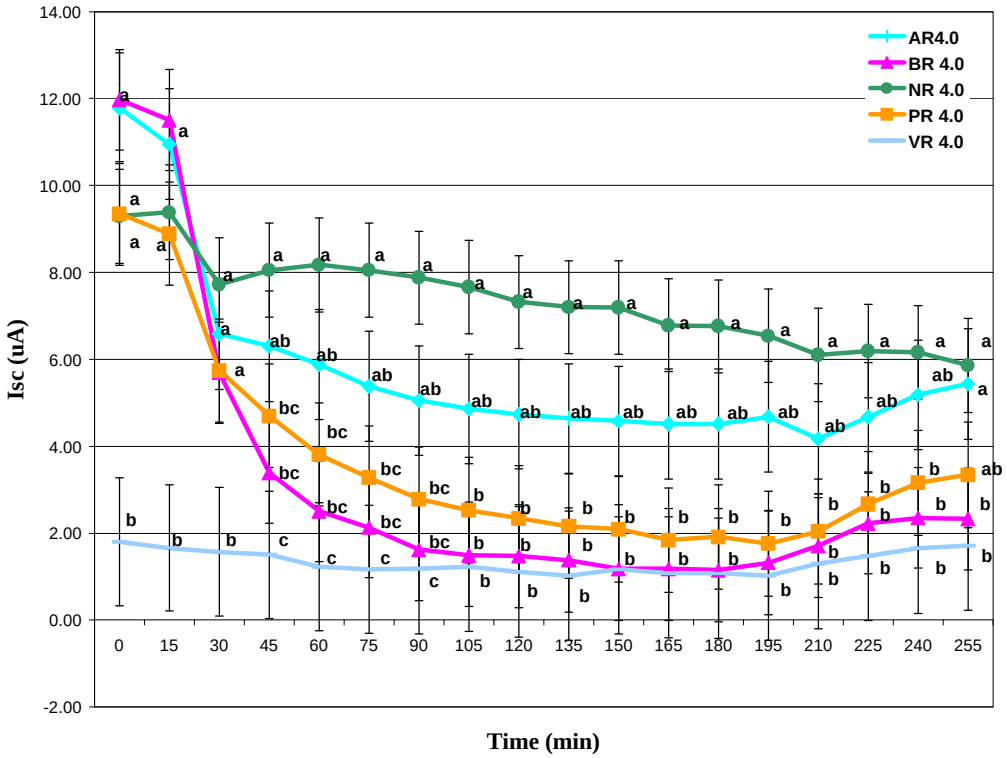


Figure 3-3. Mean Short-Circuit Current (Comparisons with VR) - pH 4.0

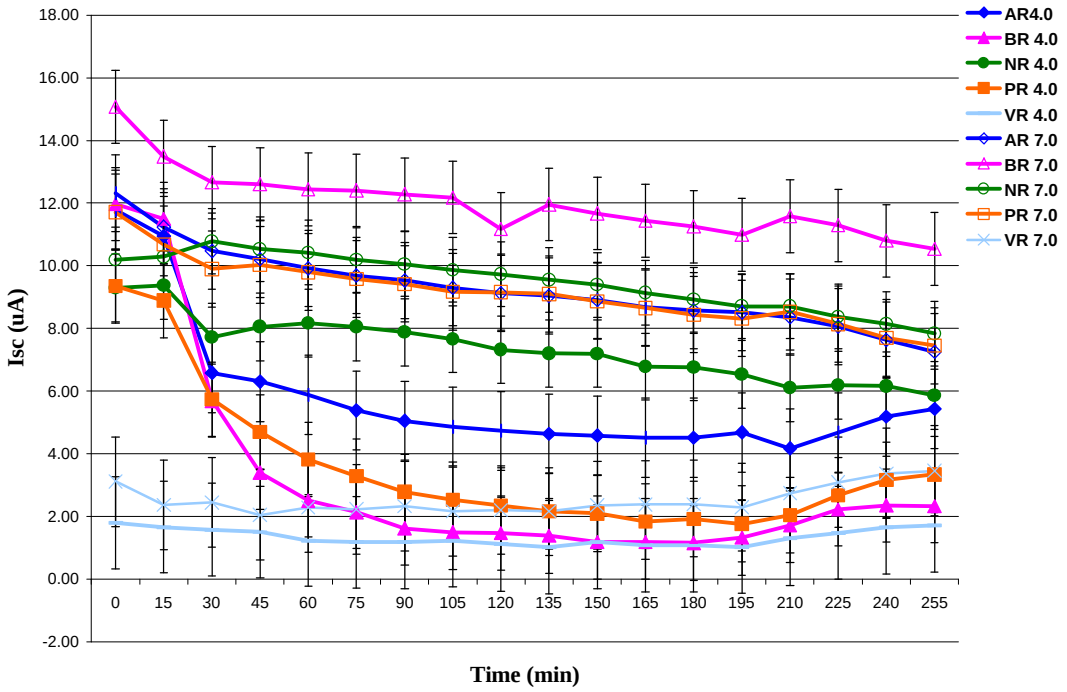


Figure 3-4. Mean Short-Circuit Current (Comparisons with VR) - pH 4.0 & 7.0

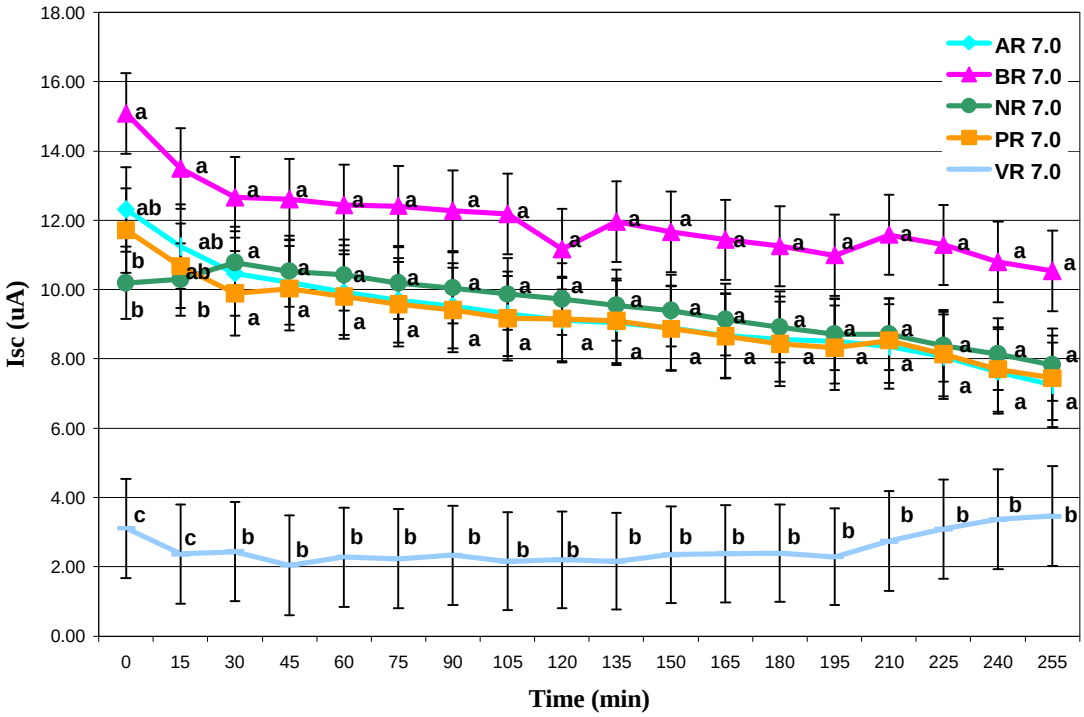


Figure 3-5. Mean Short-Circuit Current (Comparisons with VR) - pH 7.0

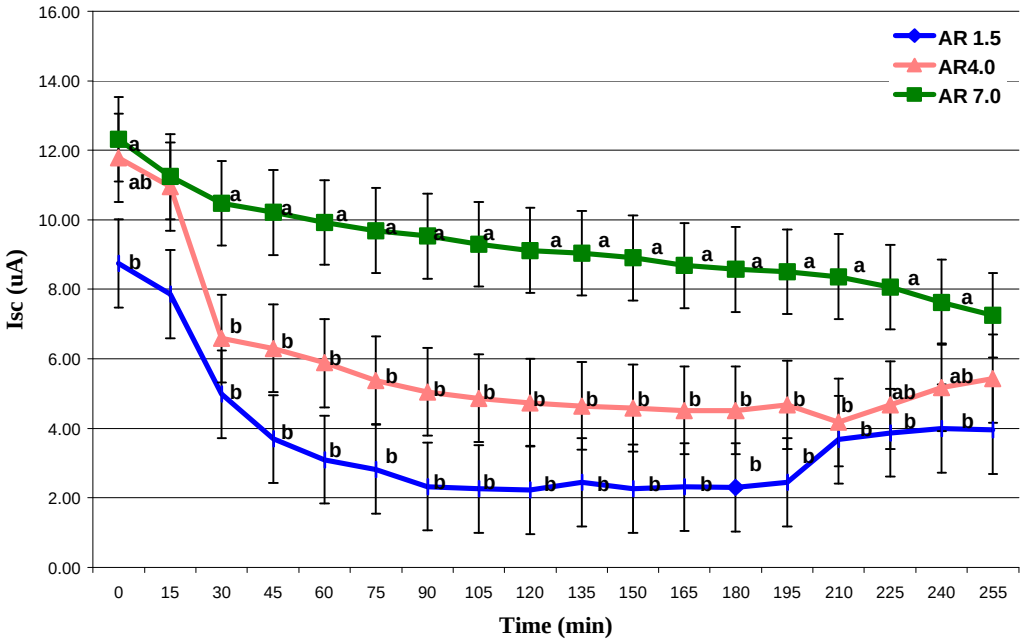


Figure 3-6. Mean Short-Circuit Current: Acetic Ringer's Solution

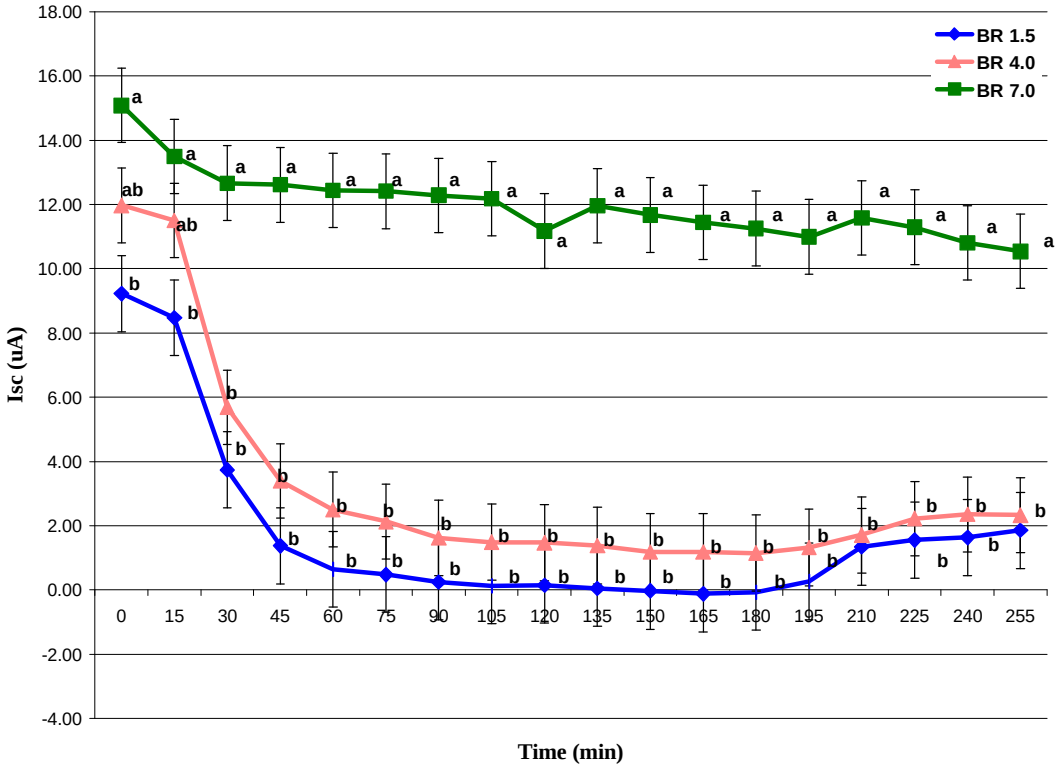


Figure 3-7. Mean Short-Circuit Current: Butyric Ringer's Solution

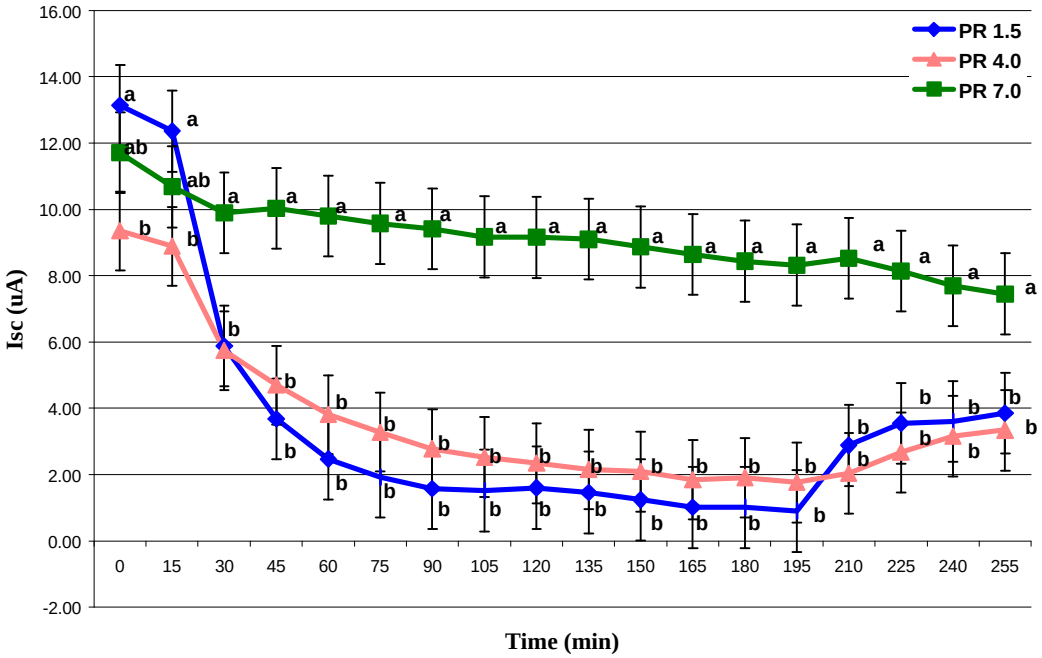


Figure 3-8. Mean Short-Circuit Current: Propionic Ringer's Solution

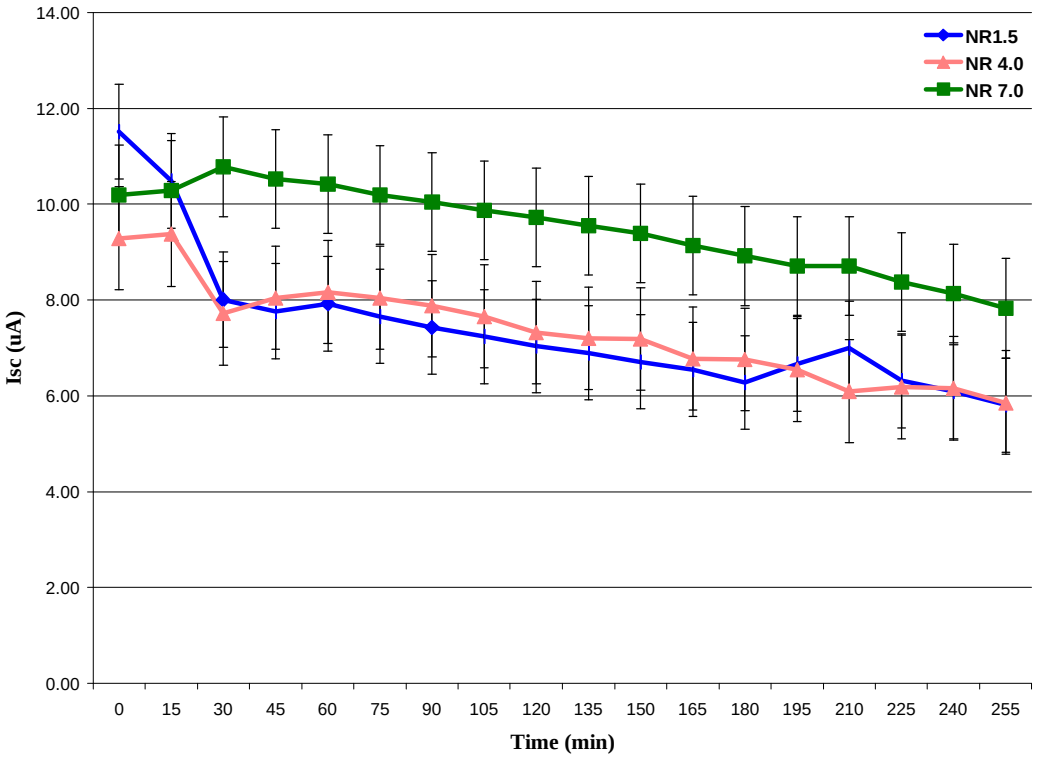


Figure 3-9. Mean Short-Circuit Current: Normal Ringer's Solution

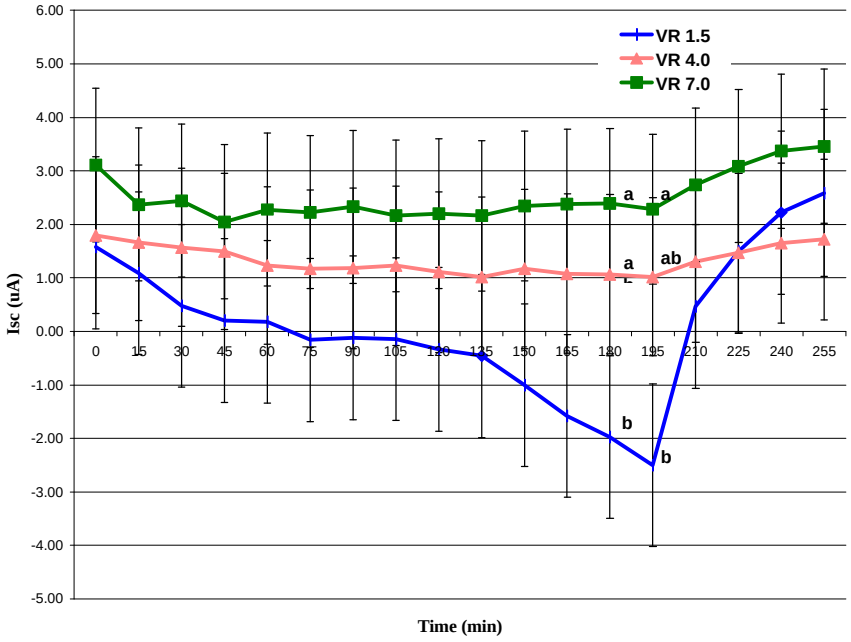


Figure 3-10. Mean Short-Circuit Current: Valeric Ringer's Solution

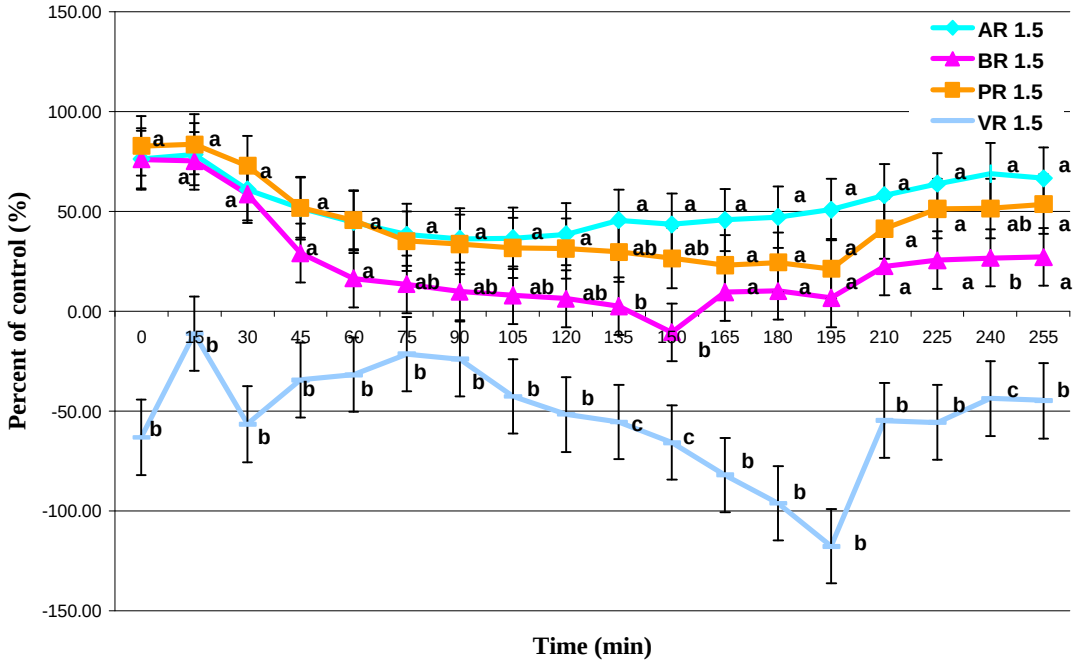


Figure 3-11. Mean Short-Circuit Current Percentage of Control (Comparisons with VR) - pH 1.5

Table 3-3. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on percentage of short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (%) ±SEM @ pH 1.5	Mean (%) ±SEM @ pH 4.0	Mean (%) ±SEM @ pH 7.0
0	Acetic (n=25,22,27)	76.30 ± 15.47^{ag}	87.00 ± 16.16^{ag}	90.53 ± 15.13^{ag}
0	Butyric (n=28,27,29)	76.16 ± 14.43^{ag}	84.69 ± 14.90^{ag}	87.64 ± 14.39^{ag}
0	Propionic (n=28,26,26)	82.88 ± 14.86^{ag}	83.30 ± 15.18^{ag}	87.88 ± 15.18^{ag}
0	Valeric (n=14,12,12)	-63.22 ± 18.92^{bdg}	30.53 ± 22.04^{beg}	33.96 ± 20.14^{beg}
15	Acetic (n=23,19,25)	78.71 ± 15.57^{a}	83.45 ± 16.36^{a}	89.09 ± 15.22^{a}
15	Butyric (n=27,25,27)	75.35 ± 14.47^{a}	82.76 ± 14.99^{a}	87.05 ± 14.47^{a}
15	Propionic (n=25,23,26)	83.66 ± 14.99^{a}	81.44 ± 15.32^{a}	86.70 ± 15.18^{a}
15	Valeric (n=16,11,13)	-11.23 ± 18.17^{b}	26.19 ± 22.12^{b}	27.34 ± 19.99^{b}
30	Acetic (n=24,22,25)	61.08 ± 15.52^{a}	78.44 ± 16.16	86.98 ± 15.22^{a}
30	Butyric (n=26,27,27)	58.73 ± 14.52^{a}	69.54 ± 14.90	89.44 ± 14.47^{a}
30	Propionic (n=25,26,25)	72.84 ± 14.99^{a}	76.12 ± 15.18	81.19 ± 15.22^{a}
30	Valeric (n=12,11,14)	-56.60 ± 19.20^{bd}	34.82 ± 22.24^{e}	23.19 ± 19.86^{be}
45	Acetic (n=22,23,25)	51.67 ± 15.63^{a}	72.58 ± 16.10	85.58 ± 15.22^{a}
45	Butyric (n=23,27,28)	29.13 ± 14.67^{ad}	48.95 ± 14.90^{de}	89.22 ± 14.43^{ae}
45	Propionic (n=24,27,26)	51.88 ± 15.04^{a}	60.33 ± 15.13	83.30 ± 15.18^{a}
45	Valeric (n=16,8,14)	-34.40 ± 18.71^{bd}	21.35 ± 22.90^{e}	20.52 ± 19.87^{be}
60	Acetic (n=26,23,26)	16.46 ± 14.52^{abd}	68.73 ± 16.10	85.64 ± 15.18^{a}
60	Butyric (n=26,25,29)	16.46 ± 14.52^{abd}	35.94 ± 14.99^{d}	88.02 ± 14.39^{ae}
60	Propionic (n=24,26,26)	45.55 ± 15.04^{a}	50.45 ± 15.18	83.01 ± 15.18^{a}
60	Valeric (n=17,9,13)	-31.84 ± 18.62^{bd}	19.03 ± 22.65^{de}	23.72 ± 19.99^{be}
75	Acetic (n=23,24,27)	38.33 ± 15.57^{ad}	66.84 ± 16.05^{de}	86.21 ± 15.13^{ae}
75	Butyric (n=29,26,30)	13.56 ± 14.39^{abd}	30.59 ± 14.95^{d}	88.77 ± 14.36^{ae}
75	Propionic (n=27,27,27)	35.16 ± 14.90^{ad}	47.95 ± 15.13^{de}	83.02 ± 15.13^{ae}
75	Valeric (n=16,9,13)	-21.44 ± 18.71^{b}	15.82 ± 22.65	23.97 ± 19.99^{b}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-3 (cont,). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on percentage of short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (%) ±SEM @ pH 1.5	Mean (%) ±SEM @ pH 4.0	Mean (%) ±SEM @ pH 7.0
90	Acetic (n=26,24,27)	36.27 ± 15.42^{ad}	66.03 ± 16.05^{ade}	86.38 ± 15.13^{ae}
90	Butyric (n=30,24,30)	9.85 ± 14.39^{abd}	29.62 ± 15.05^{abd}	88.97 ± 14.36^{ae}
90	Propionic (n=26,26,27)	33.67 ± 14.95^{ad}	46.53 ± 15.18^{abde}	82.71 ± 15.13^{ae}
90	Valeric (n=16,9,14)	-23.96 ± 18.71^{b}	7.19 ± 22.66^{b}	24.58 ± 19.87^{b}
105	Acetic (n=26,24,27)	36.50 ± 15.42^{ad}	66.26 ± 16.05^{ade}	85.97 ± 15.13^{ae}
105	Butyric (n=29,23,30)	8.01 ± 14.39^{abd}	28.20 ± 15.11^{abd}	89.21 ± 14.36^{ae}
105	Propionic (n=25,24,26)	31.80 ± 15.00^{ad}	44.57 ± 15.28^{abde}	82.32 ± 15.18^{ae}
105	Valeric (n=17,10,15)	-42.65 ± 18.62^{bd}	14.59 ± 22.43^{bde}	26.13 ± 19.76^{be}
120	Acetic (n=24,24,27)	38.59 ± 15.52^{ad}	68.11 ± 16.05^{de}	86.12 ± 15.13^{ae}
120	Butyric (n=29,21,29)	6.28 ± 14.39^{bd}	26.67 ± 15.23^{d}	88.52 ± 14.39^{ae}
120	Propionic (n=23,24,26)	31.43 ± 15.10^{ad}	47.00 ± 15.28^{de}	82.30 ± 15.18^{ae}
120	Valeric (n=16,10,16)	-51.71 ± 18.71^{bd}	14.71 ± 22.43^{e}	27.10 ± 19.66^{be}
135	Acetic (n=25,24,27)	45.56 ± 15.47^{ad}	68.34 ± 16.05^{de}	86.34 ± 15.13^{ae}
135	Butyric (n=28,20,29)	2.53 ± 14.43^{bd}	27.68 ± 15.30^{d}	88.85 ± 14.39^{ae}
135	Propionic (n=23,25,26)	29.67 ± 15.10^{abd}	45.57 ± 15.23^{de}	83.07 ± 15.18^{ae}
135	Valeric (n=16,10,16)	-55.51 ± 18.71^{cd}	14.39 ± 22.43^{e}	25.62 ± 19.66^{b}
150	Acetic (n=25,24,27)	43.45 ± 15.47^{ad}	68.84 ± 16.05^{e}	86.23 ± 15.13^{ae}
150	Butyric (n=27,23,29)	-10.58 ± 14.43^{bd}	27.91 ± 15.11^{d}	88.58 ± 14.39^{ae}
150	Propionic (n=26,24,26)	26.58 ± 14.95^{abd}	43.92 ± 15.28^{de}	82.51 ± 15.18^{ae}
150	Valeric (n=17,10,14)	-65.81 ± 18.62^{cd}	15.40 ± 22.43^{e}	26.69 ± 19.86^{be}
165	Acetic (n=26,24,27)	45.71 ± 15.42^{a}	69.73 ± 16.05	85.92 ± 15.13^{a}
165	Butyric (n=26,21,29)	9.57 ± 14.52^{ad}	28.28 ± 15.23^{d}	88.80 ± 14.39^{ae}
165	Propionic (n=25,25,26)	23.16 ± 15.00^{ad}	43.74 ± 15.23^{de}	82.64 ± 15.18^{ae}
165	Valeric (n=17,10,16)	-81.99 ± 18.62^{bd}	16.34 ± 12.43^{e}	27.97 ± 19.66^{be}
180	Acetic (n=25,24,27)	47.17 ± 15.47^{a}	70.22 ± 16.05	86.09 ± 15.13^{a}
180	Butyric (n=28,21,29)	10.14 ± 14.43^{ad}	28.35 ± 15.23^{d}	88.50 ± 14.39^{ae}
180	Propionic (n=25,25,26)	24.51 ± 14.99^{ad}	43.67 ± 15.23^{de}	82.94 ± 15.18^{ae}
180	Valeric (n=17,10,16)	-96.17 ± 18.62^{bd}	16.24 ± 22.43^{e}	29.15 ± 19.66^{be}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH;^{gh}value differs from initial value after change to NR solution

Table 3-3 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on percentage of short-circuit current in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (%) ±SEM @ pH 1.5	Mean (%) ±SEM @ PH 4.0	Mean (%) ±SEM @ pH 7.0
195	Acetic (n=25,22,25)	50.92 ± 15.47[a]	70.74 ± 16.16	86.36 ± 15.22[a]
195	Butyric (n=24,20,26)	6.71 ± 14.62[ad]	28.67 ± 15.30[d]	89.44 ± 14.52[ae]
195	Propionic (n=24,24,25)	21.30 ± 15.04[ad]	45.56 ± 15.28[de]	83.68 ± 15.22[ae]
195	Valeric (n=16,10,16)	-117.78 ± 18.71[bd]	16.26 ± 22.43[e]	32.66 ± 19.66[be]
210	Acetic (n=25,24,27)	58.18 ± 15.47[a]	70.82 ± 16.05	84.93 ± 15.13[a]
210	Butyric (n=26,21,29)	22.59 ± 14.52[ad]	35.46 ± 15.23[d]	91.43 ± 14.39[ae]
210	Propionic (n=23,23,25)	41.31 ± 15.10[ad]	50.32 ± 15.33[de]	85.77 ± 15.22[ae]
210	Valeric (n=16,8,13)	-54.71 ± 18.71[bd]	18.28 ± 22.93[e]	38.99 ± 19.99[be]
225	Acetic (n=26,24,27)	63.88 ± 15.42[a]	75.06 ± 16.05	86.05 ± 15.13[a]
225	Butyric (n=27,25,29)	25.54 ± 14.47[ad]	39.67 ± 15.00[d]	91.56 ± 14.39[ae]
225	Propionic (n=26,23,26)	51.37 ± 14.95[a]	58.34 ± 15.33	86.21 ± 15.18[a]
225	Valeric (n=15,9,14)	-55.67 ± 18.81[bd]	21.52 ± 22.65[e]	46.70 ± 19.87[be]
240	Acetic (n=25,24,27)	68.97 ± 15.47[a]	78.30 ± 16.05	84.94 ± 15.13[a]
240	Butyric (n=29,25,29)	26.70 ± 14.39[bd]	43.70 ± 15.00[d]	90.74 ± 14.39[ae]
240	Propionic (n=27,23,26)	51.55 ± 14.90[ab]	61.79 ± 15.33	85.14 ± 15.18[a]
240	Valeric (n=16,8,13)	-43.72 ± 18.71[cd]	21.66 ± 22.93[e]	45.61 ± 19.99[be]
255	Acetic (n=24,24,25)	66.61 ± 15.52[ag]	80.35 ± 16.05[ag]	84.19 ± 15.22[ag]
255	Butyric (n=26,24,27)	27.15 ± 14.52[adh]	46.39 ± 15.05[abdh]	90.44 ± 14.47[aeg]
255	Propionic (n=27,24,26)	53.67 ± 14.90[ah]	62.88 ± 15.28[abh]	85.20 ± 15.18[ag]
255	Valeric (n=14,7,13)	-44.79 ± 18.92[bdg]	23.16 ± 23.28[abeg]	44.48 ± 19.99[beg]

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); [abc]for differences within treatment, [def]for differences within pH; [gh]value differs from initial value after change to NR solution

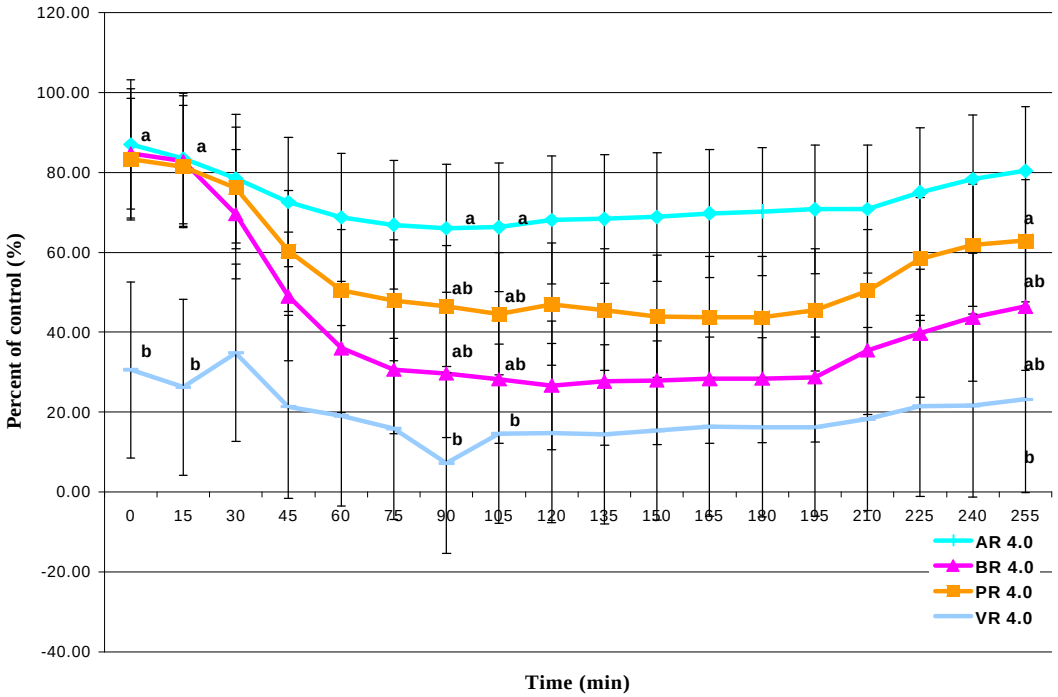


Figure 3-12 Mean Short-Circuit Current Percentage of Control (Comparisons with VR) - pH 4.0

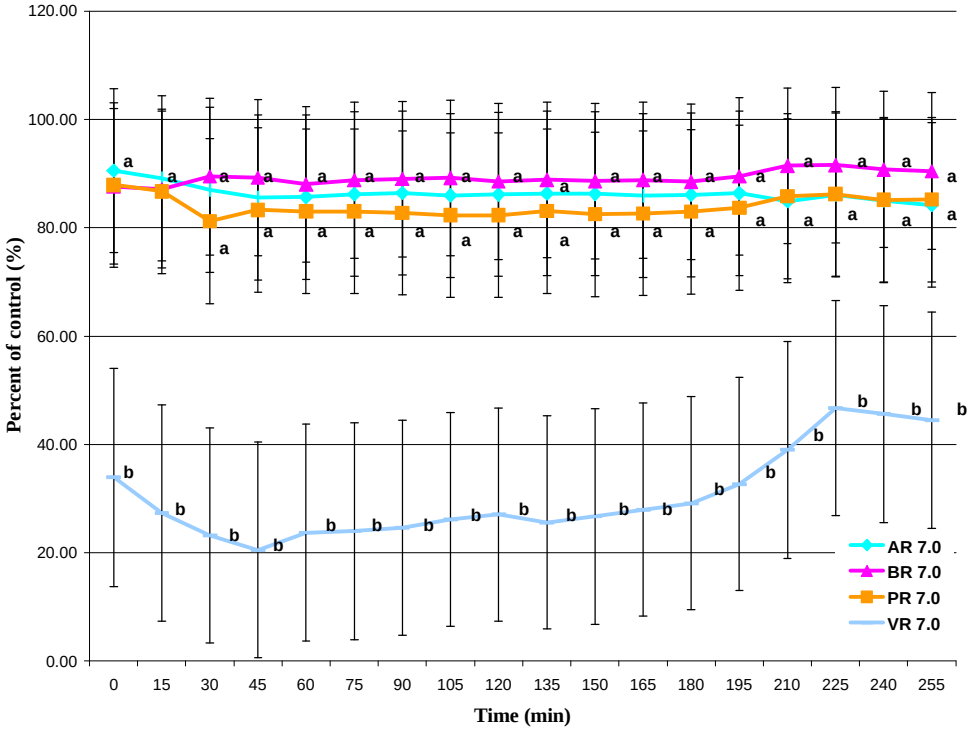


Figure 3-13. Mean Short-Circuit Current Percentage of Control (Comparisons with VR) - pH 7.0

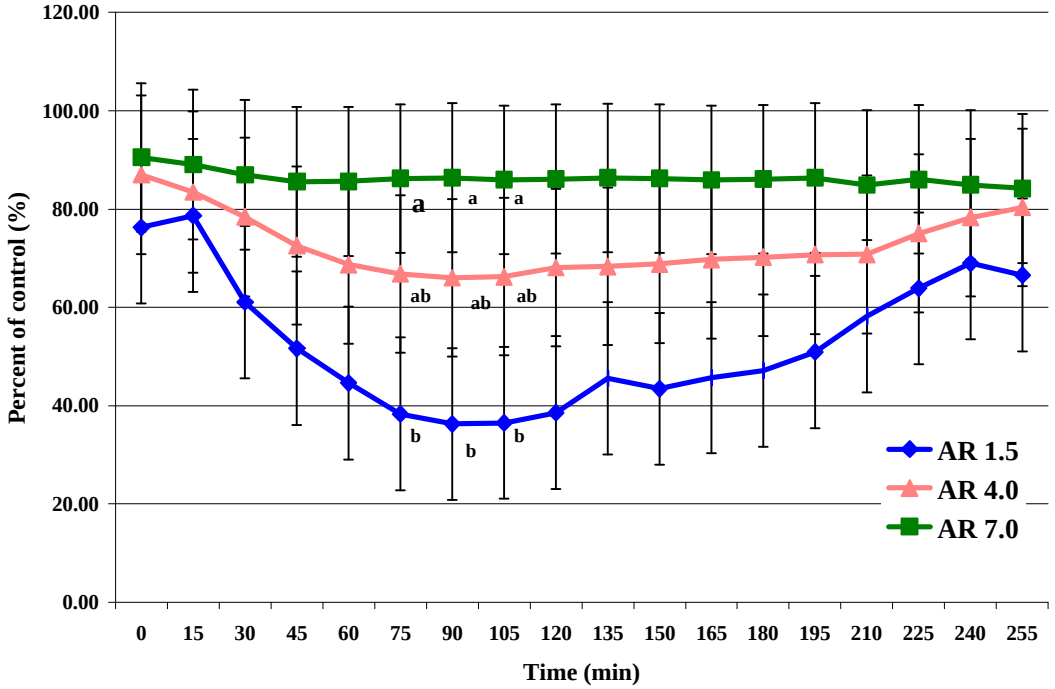


Figure 3-14. Mean Short-Circuit Current Percentage of Control: Acetic Ringer's Solution

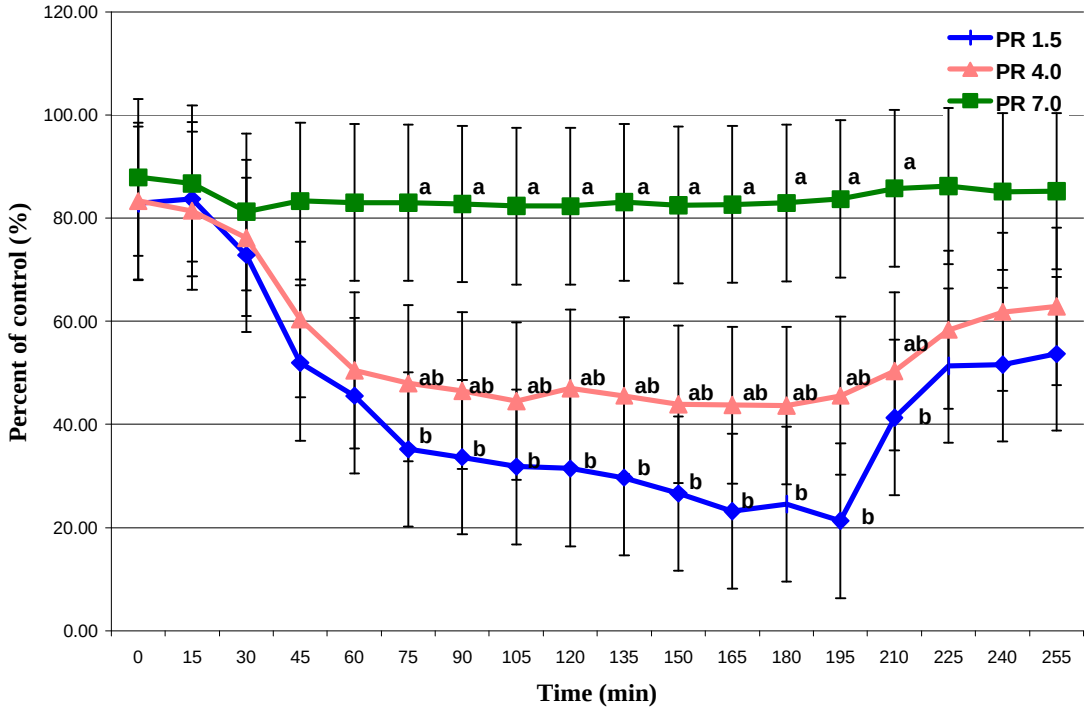


Figure 3-15. Mean Short-Circuit Current Percentage of Control: Propionic Ringer's Solution

at pH 1.5 and 4.0 compared to pH 7.0 (Figure 3-16). Valeric acid decreased ($P<0.01$) PCIsc at pH 1.5 compared to pH 4.0 and 7.0 (Figure 3-17).

Butyric acid and propionic acid decreased ($P<0.01$) PD following the 30 min acclimation period at pH 1.5. Valeric acid also decreased ($P<0.01$) PD following the 30 min acclimation period at pH 1.5 (Figure 3-18, Table 3-4). There was some, but not complete recovery in butyric and propionic acid exposed tissues after 195 min of exposure. Butyric acid decreased ($P<0.01$) PD following the 30 min acclimation period at pH 4.0. Valeric acid also decreased ($P<0.01$) PD following the 30 min acclimation period at pH 4.0. (Figure 3-19, Table 3-4). There was some, but not complete recovery in butyric and acid exposed tissues after 195 min of exposure. PD was decreased ($P<0.01$) the greatest in butyric and valeric acid followed by propionic acid. Valeric acid decreased ($P<0.01$) PD following the 30 min acclimation period at pH 7.0 (Figure 3-20, Table 3-4).

Acetic acid and normal Ringer's exposed tissues decreased ($P<0.01$) PD at pH 1.5 compared to pH 4.0 and 7.0 (Figure 3-21, 3-22). Butyric acid decreased ($P<0.01$) PD at pH 1.5 and 4.0 compared to pH 7.0 (Figure 3-23). Propionic acid decreased ($P<0.01$) PD the greatest at pH 1.5 followed by pH 4.0 (Figure 3-24). Valeric acid showed a trend toward decreasing PD at pH 1.5 compared to pH 4.0 and 7.0.

Valeric acid decreased ($P<0.01$) R after the first 105 min at pH 1.5 (Figure 3-26, Table 3-5). Valeric acid was higher and decreased ($P<0.01$) in R at pH 4.0 and pH 7.0 (Figure 3-27, 3-28, Table 3-5).

There was a trend for acetic acid to decrease R the greatest at pH 7.0 followed by pH 4.0 (Figure 3-29). There was a trend for butyric acid to decrease R at pH 1.5

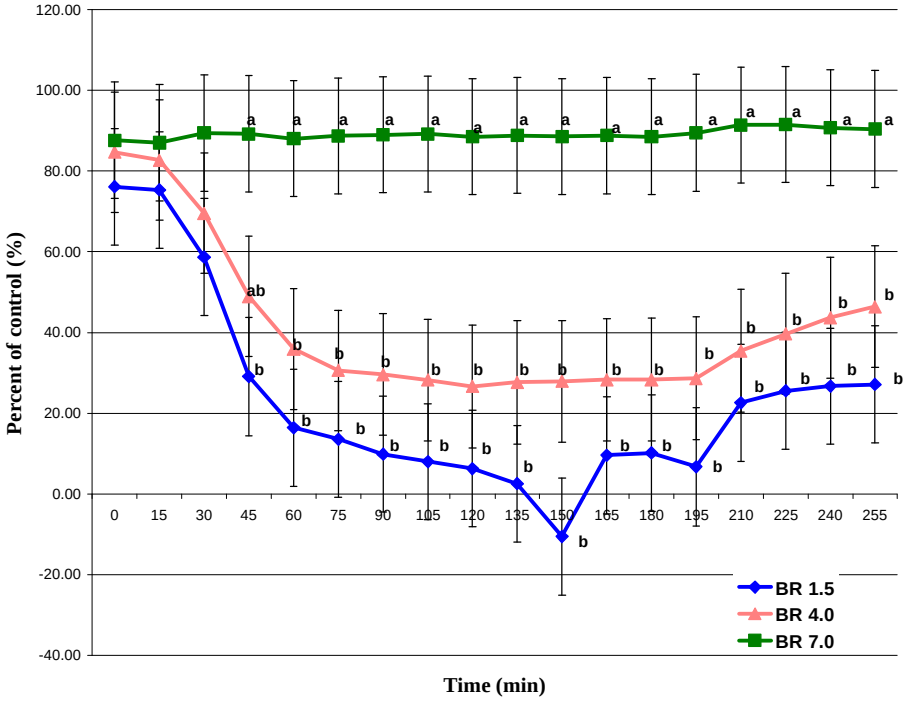


Figure 3-16. Mean Short-Circuit Current Percentage of Control: Butyric Ringer's Solution

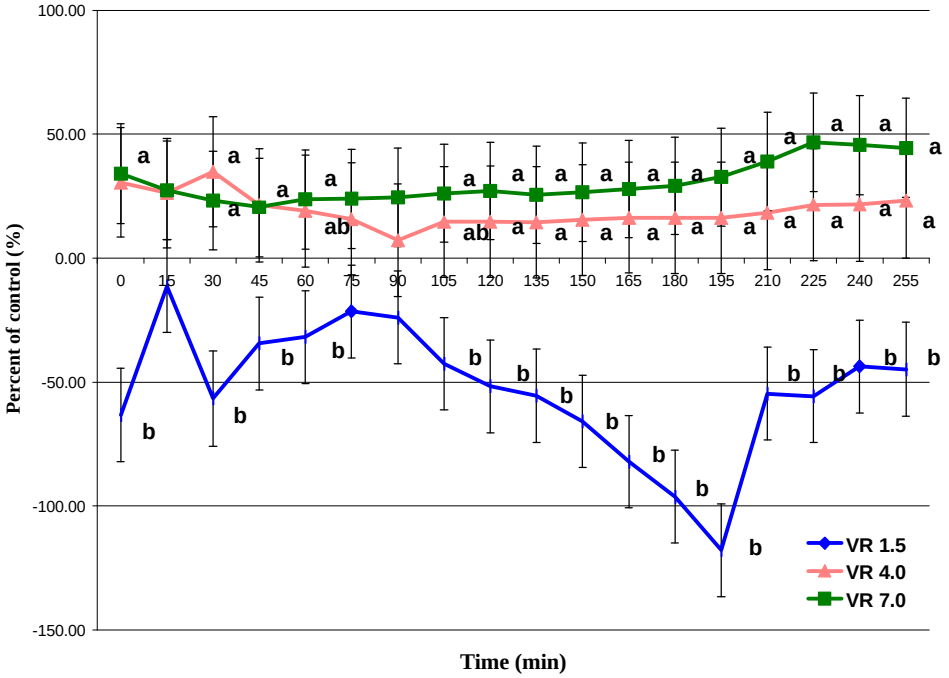


Figure 3-17. Mean Short-Circuit Current Percentage of Control: Valeric Ringer's Solution

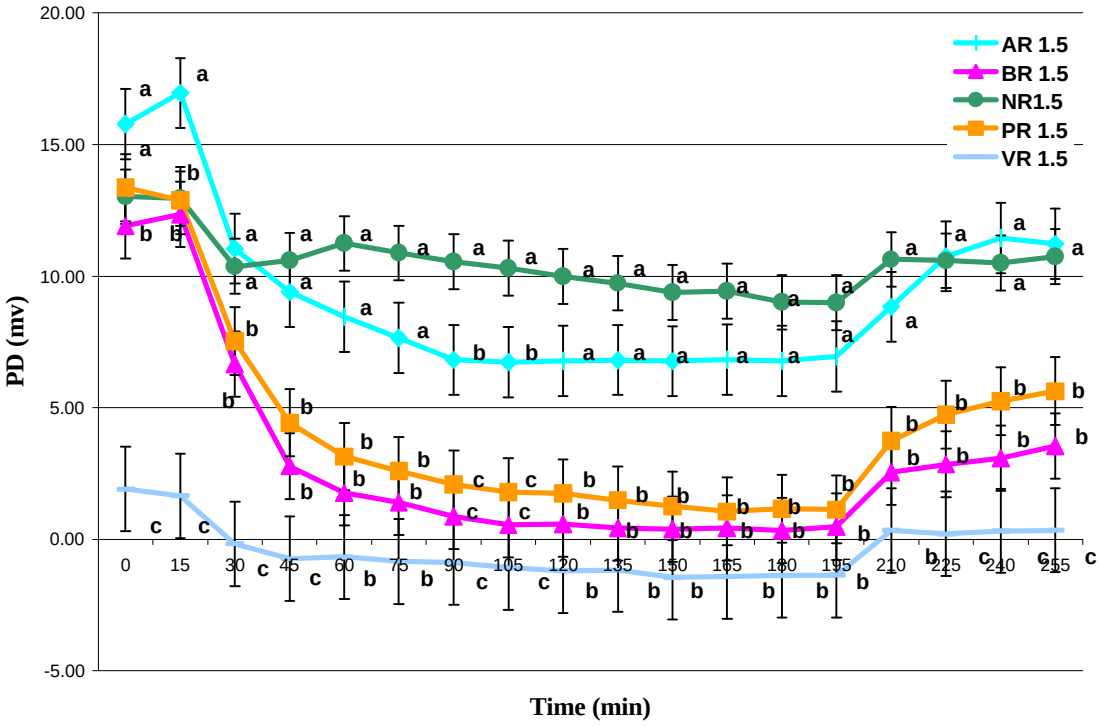


Figure 3-18. Mean PD (Comparisons with VR) - pH 1.5

Table 3-4. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mv) ±SEM @ pH 1.5	Mean (mv) ±SEM @ pH 4.0	Mean (mv) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	15.78 ± 1.34^{ag}	16.06 ± 1.34^{ag}	13.83 ± 1.29^{ag}
0	Butyric (n=29,29,29)	11.90 ± 1.24^{bg}	12.85 ± 1.23^{ag}	14.65 ± 1.23^{ag}
0	Normal (n=40,35,38)	13.02 ± 1.04^{ag}	13.41 ± 1.13^{ag}	12.69 ± 1.09^{ag}
0	Propionic (n=28,29,28)	13.36 ± 1.28^{ag}	13.92 ± 1.26^{ag}	13.52 ± 1.28^{ag}
0	Valeric (n=18,19,20)	1.91 ± 1.60^{cg}	2.28 ± 1.53^{bg}	2.08 ± 1.48^{bg}
15	Acetic (n=26,25,28)	16.96 ± 1.33^{ad}	15.18 ± 1.34^{ade}	13.83 ± 1.29^{a}
15	Butyric (n=30,31,29)	12.34 ± 1.24^{b}	12.98 ± 1.22^{a}	14.38 ± 1.23^{a}
15	Normal (n=41,34,39)	12.95 ± 1.04^{b}	13.64 ± 1.14^{a}	13.07 ± 1.09^{a}
15	Propionic (n=28,29,28)	12.88 ± 1.28^{b}	13.48 ± 1.26^{a}	13.43 ± 1.28^{a}
15	Valeric (n=18,20,21)	1.65 ± 1.60^{c}	2.17 ± 1.52^{b}	1.38 ± 1.48^{b}
30	Acetic (n=26,25,28)	11.05 ± 1.33^{a}	11.32 ± 1.34^{a}	13.72 ± 1.28^{a}
30	Butyric (n=29,31,30)	6.66 ± 1.24^{bd}	8.19 ± 1.22^{bd}	14.19 ± 1.22^{ae}
30	Normal (n=38,35,39)	10.37 ± 1.05^{ad}	12.15 ± 1.13^{ade}	13.80 ± 1.09^{ae}
30	Propionic (n=27,29,27)	7.54 ± 1.29^{bd}	10.52 ± 1.26^{ade}	12.91 ± 1.29^{ae}
30	Valeric (n=17,20,19)	-0.17 ± 1.61^{c}	2.39 ± 1.52^{c}	1.66 ± 1.49^{b}
45	Acetic (n=25,25,27)	9.40 ± 1.34^{ad}	12.10 ± 1.34^{ade}	13.77 ± 1.28^{ae}
45	Butyric (n=28,31,31)	2.77 ± 1.25^{bd}	5.93 ± 1.22^{bd}	14.18 ± 1.22^{ae}
45	Normal (n=42,35,39)	10.60 ± 1.04^{ad}	12.69 ± 1.13^{ade}	13.90 ± 1.09^{ae}
45	Propionic (n=28,29,28)	4.43 ± 1.28^{bd}	9.50 ± 1.26^{ae}	13.36 ± 1.28^{af}
45	Valeric (n=18,20,19)	-0.74 ± 1.60^{c}	1.79 ± 1.52^{c}	1.37 ± 1.50^{b}
60	Acetic (n=26,26,28)	8.46 ± 1.33^{ad}	12.23 ± 1.33^{ae}	13.90 ± 1.28^{ae}
60	Butyric (n=29,31,31)	1.76 ± 1.24^{bd}	4.11 ± 1.22^{cd}	14.34 ± 1.22^{ae}
60	Normal (n=40,34,39)	11.25 ± 1.04^{a}	13.38 ± 1.14^{a}	13.90 ± 1.09^{ae}
60	Propionic (n=28,29,28)	3.15 ± 1.28^{bd}	8.03 ± 1.26^{be}	13.05 ± 1.28^{af}
60	Valeric (n=18,20,19)	-0.67 ± 1.60^{b}	1.71 ± 1.52^{c}	1.79 ± 1.50^{b}
75	Acetic (n=25,26,28)	7.65 ± 1.34^{ad}	12.07 ± 1.33^{ae}	14.06 ± 1.28^{ae}
75	Butyric (n=30,31,31)	1.40 ± 1.24^{bd}	2.63 ± 1.22^{cd}	14.91 ± 1.22^{ae}
75	Normal (n=43,35,39)	10.88 ± 1.04^{ad}	13.59 ± 1.13^{ade}	14.17 ± 1.09^{ae}
75	Propionic (n=27,29,28)	2.59 ± 1.29^{bd}	7.39 ± 1.26^{be}	13.19 ± 1.28^{af}
75	Valeric (n=17,20,19)	-0.84 ± 1.61^{b}	1.67 ± 1.52^{c}	1.55 ± 1.50^{b}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-4 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mv) ±SEM @ pH 1.5	Mean (mv) ±SEM @ pH 4.0	Mean (mv) ±SEM @ pH 7.0
90	Acetic (n=26,26,28)	6.82 ± 1.33^{bd}	12.29 ± 1.33^{ae}	14.19 ± 1.28^{ae}
90	Butyric (n=30,31,31)	0.86 ± 1.24^{cd}	3.36 ± 1.22^{cd}	14.68 ± 1.22^{ae}
90	Normal (n=43,36,39)	10.55 ± 1.04^{ad}	13.41 ± 1.13^{ade}	14.31 ± 1.09^{a}
90	Propionic (n=27,29,28)	2.08 ± 1.29^{cd}	7.00 ± 1.26^{be}	13.14 ± 1.28^{af}
90	Valeric (n=18,20,20)	-0.89 ± 1.60^{c}	1.67 ± 1.52^{c}	1.70 ± 1.50^{b}
105	Acetic (n=26,26,28)	6.73 ± 1.33^{bd}	11.77 ± 1.33^{ae}	14.16 ± 1.28^{ae}
105	Butyric (n=29,31,31)	0.54 ± 1.24^{cd}	2.63 ± 1.22^{cd}	14.91 ± 1.22^{ae}
105	Normal (n=43,36,39)	10.30 ± 1.04^{ad}	13.35 ± 1.13^{ade}	14.35 ± 1.09^{ae}
105	Propionic (n=26,29,28)	1.80 ± 1.29^{cd}	6.65 ± 1.26^{be}	13.05 ± 1.28^{af}
105	Valeric (n=18,20,21)	-1.08 ± 1.60^{c}	1.72 ± 1.52^{c}	1.59 ± 1.49^{b}
120	Acetic (n=26,26,28)	6.78 ± 1.33^{ad}	11.85 ± 1.33^{ae}	14.16 ± 1.28^{ae}
120	Butyric (n=29,30,30)	0.58 ± 1.24^{bd}	2.47 ± 1.22^{cd}	14.90 ± 1.22^{ae}
120	Normal (n=43,36,39)	9.99 ± 1.04^{ad}	13.28 ± 1.13^{ae}	14.59 ± 1.09^{ae}
120	Propionic (n=26,29,28)	1.74 ± 1.29^{bd}	6.63 ± 1.26^{be}	12.64 ± 1.28^{af}
120	Valeric (n=18,20,21)	-1.19 ± 1.60^{b}	1.75 ± 1.52^{c}	1.40 ± 1.48^{b}
135	Acetic (n=26,26,28)	6.81 ± 1.33^{ad}	12.29 ± 1.33^{ae}	14.28 ± 1.28^{ae}
135	Butyric (n=29,30,30)	0.43 ± 1.24^{bd}	2.39 ± 1.22^{cd}	14.98 ± 1.22^{ae}
135	Normal (n=43,36,39)	9.73 ± 1.04^{ad}	13.23 ± 1.13^{ae}	14.61 ± 1.09^{ae}
135	Propionic (n=26,29,28)	1.49 ± 1.29^{bd}	6.47 ± 1.26^{be}	12.19 ± 1.28^{af}
135	Valeric (n=18,20,21)	-1.17 ± 1.60^{b}	1.76 ± 1.52^{c}	1.47 ± 1.48^{b}
150	Acetic (n=26,26,28)	6.77 ± 1.33^{ad}	11.99 ± 1.33^{ae}	14.28 ± 1.28^{ae}
150	Butyric (n=29,31,30)	0.39 ± 1.24^{bd}	2.36 ± 1.22^{cd}	15.06 ± 1.22^{ae}
150	Normal (n=43,36,39)	9.39 ± 1.04^{ad}	13.31 ± 1.13^{ae}	14.61 ± 1.09^{ae}
150	Propionic (n=27,29,28)	1.27 ± 1.29^{bd}	6.29 ± 1.26^{be}	12.11 ± 1.28^{af}
150	Valeric (n=18,20,21)	-1.45 ± 1.60^{b}	1.82 ± 1.52^{c}	1.67 ± 1.48^{b}
165	Acetic (n=26,26,28)	6.83 ± 1.33^{ad}	12.11 ± 1.33^{ae}	14.21 ± 1.28^{ae}
165	Butyric (n=29,31,30)	0.43 ± 1.24^{bd}	2.41 ± 1.22^{cd}	14.98 ± 1.22^{ae}
165	Normal (n=43,36,39)	9.43 ± 1.04^{ad}	12.99 ± 1.13^{ae}	14.62 ± 1.09^{ae}
165	Propionic (n=27,29,28)	1.05 ± 1.29^{bd}	6.01 ± 1.26^{be}	11.98 ± 1.28^{af}
165	Valeric (n=18,20,21)	-1.42 ± 1.60^{b}	1.91 ± 1.52^{c}	2.16 ± 1.48^{b}
180	Acetic (n=26,26,28)	6.78 ± 1.33^{ad}	12.25 ± 1.33^{ae}	14.19 ± 1.28^{ae}
180	Butyric (n=29,31,30)	0.34 ± 1.24^{bd}	2.35 ± 1.22^{cd}	14.95 ± 1.22^{ae}
180	Normal (n=43,36,39)	9.01 ± 1.04^{ad}	13.17 ± 1.13^{ae}	14.61 ± 1.09^{ae}
180	Propionic (n=26,29,28)	1.16 ± 1.29^{bd}	5.89 ± 1.26^{be}	11.87 ± 1.28^{af}
180	Valeric (n=18,20,21)	-1.38 ± 1.60^{b}	1.86 ± 1.52^{c}	2.12 ± 1.48^{b}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-4 (cont). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on potential difference in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mv) ±SEM @ pH 1.5	Mean (mv) ±SEM @ pH 4.0	Mean (mv) ±SEM @ pH 7.0
195	Acetic (n=25,24,26)	6.95 ± 1.34^{ad}	12.14 ± 1.34^{ae}	14.25 ± 1.29^{ae}
195	Butyric (n=25,29,27)	0.48 ± 1.26^{bd}	2.49 ± 1.23^{bd}	14.91 ± 1.23^{ae}
195	Normal (n=41,35,38)	8.99 ± 1.04^{ad}	12.92 ± 1.13^{ae}	14.49 ± 1.09^{ae}
195	Propionic (n=27,28,27)	1.13 ± 1.29^{bd}	5.92 ± 1.26^{be}	11.78 ± 1.29^{af}
195	Valeric (n=18,20,21)	-1.36 ± 1.60^{b}	1.83 ± 1.52^{b}	2.08 ± 1.48^{b}
210	Acetic (n=26,26,28)	8.84 ± 1.33^{ad}	11.02 ± 1.33^{ade}	13.83 ± 1.28^{ae}
210	Butyric (n=28,31,30)	2.55 ± 1.25^{bd}	2.72 ± 1.22^{cd}	14.78 ± 1.22^{ae}
210	Normal (n=43,35,39)	10.64 ± 1.04^{ad}	12.32 ± 1.13^{ade}	14.58 ± 1.09^{ae}
210	Propionic (n=26,29,28)	3.74 ± 1.29^{bd}	6.33 ± 1.26^{bd}	11.46 ± 1.28^{ae}
210	Valeric (n=18,20,21)	0.34 ± 1.60^{b}	2.04 ± 1.52^{c}	2.80 ± 1.48^{b}
225	Acetic (n=26,26,28)	10.75 ± 1.33^{a}	12.43 ± 1.33^{a}	13.19 ± 1.28^{ae}
225	Butyric (n=28,31,31)	2.85 ± 1.25^{bd}	3.42 ± 1.22^{cd}	14.70 ± 1.22^{ae}
225	Normal (n=43,35,39)	10.59 ± 1.04^{ad}	12.53 ± 1.13^{ade}	14.17 ± 1.09^{ae}
225	Propionic (n=27,29,28)	4.74 ± 1.29^{bd}	7.78 ± 1.26^{bde}	10.95 ± 1.28^{ae}
225	Valeric (n=18,20,21)	0.21 ± 1.60^{c}	2.22 ± 1.52^{c}	2.77 ± 1.48^{b}
240	Acetic (n=25,26,28)	10.75 ± 1.33^{a}	13.50 ± 1.33^{a}	12.95 ± 1.28^{a}
240	Butyric (n=30,31,31)	3.08 ± 1.24^{bd}	3.81 ± 1.22^{cd}	14.26 ± 1.22^{ae}
240	Normal (n=43,35,39)	10.50 ± 1.04^{ad}	12.42 ± 1.13^{ade}	13.99 ± 1.09^{ae}
240	Propionic (n=27,29,28)	5.25 ± 1.29^{bd}	8.73 ± 1.26^{bde}	10.45 ± 1.28^{ae}
240	Valeric (n=18,20,21)	0.32 ± 1.60^{c}	2.31 ± 1.52^{c}	2.77 ± 1.48^{b}
255	Acetic (n=24,24,26)	11.22 ± 1.34^{ah}	13.71 ± 1.34^{ah}	12.27 ± 1.29^{ah}
255	Butyric (n=28,30,29)	3.54 ± 1.25^{bdh}	5.38 ± 1.22^{cdh}	14.12 ± 1.23^{aeg}
255	Normal (n=42,34,38)	10.74 ± 1.04^{adh}	12.04 ± 1.14^{abdeh}	13.70 ± 1.09^{aeh}
255	Propionic (n=27,27,27)	5.63 ± 1.29^{bdh}	9.40 ± 1.27^{beh}	10.54 ± 1.29^{aeh}
255	Valeric (n=18,20,20)	0.35 ± 1.60^{cg}	2.35 ± 1.52^{cg}	2.77 ± 1.49^{bg}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within H; ^{gh}value differs from initial value after change to NR solution

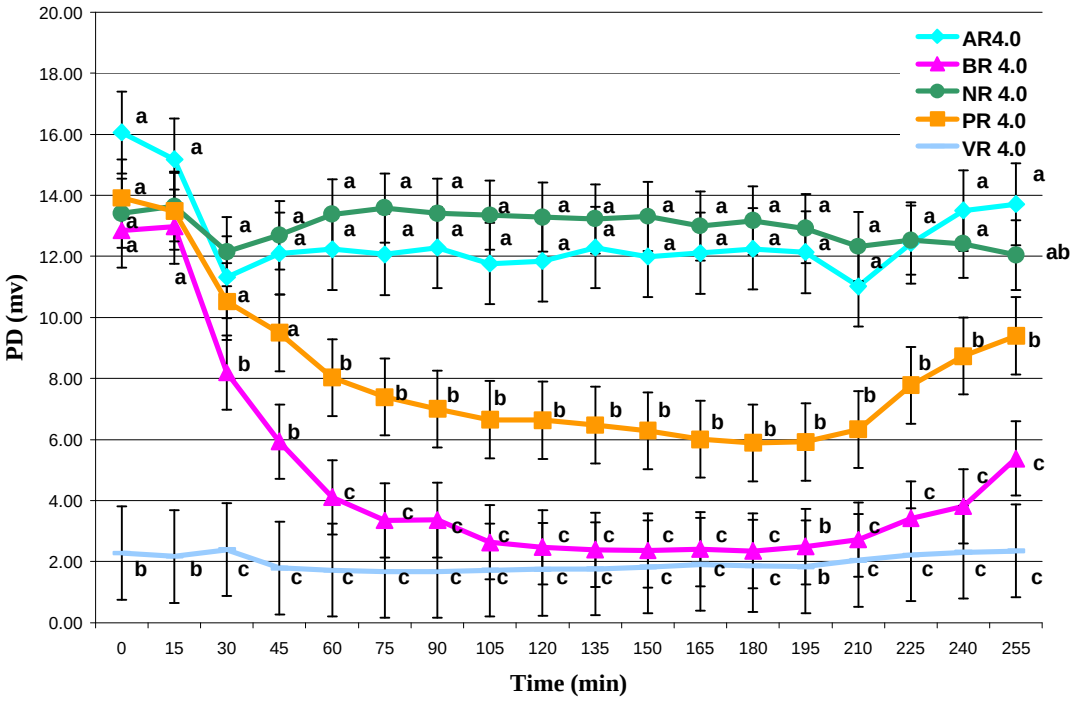


Figure 3-19. Mean PD (Comparisons with VR) - pH 4.0

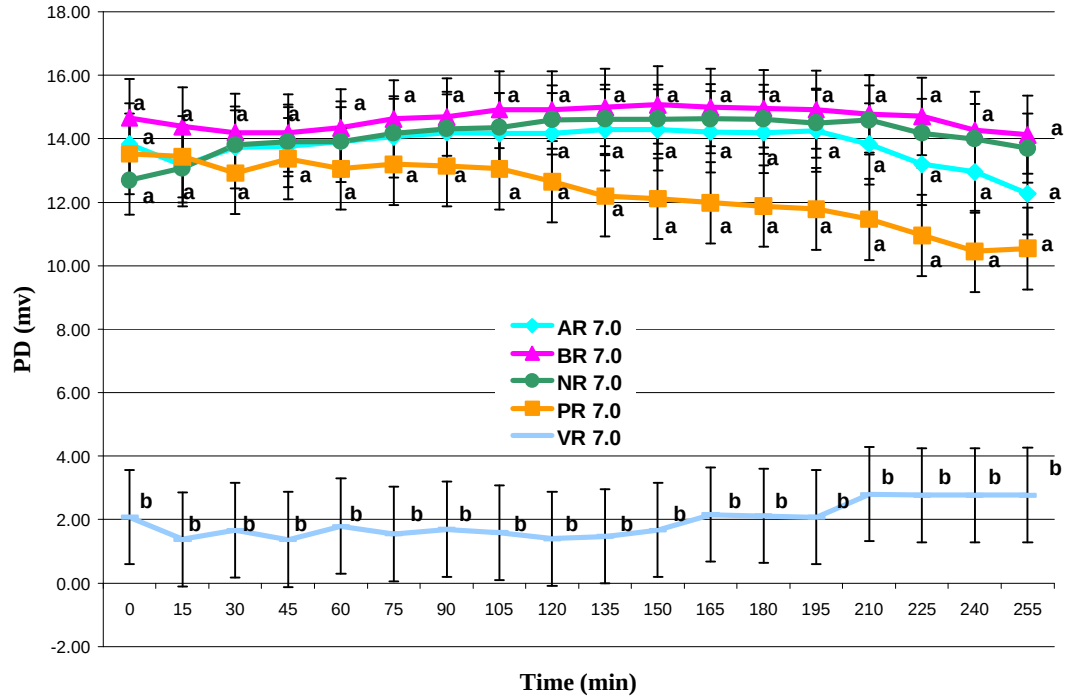


Figure 3-20. Mean PD (Comparisons with VR) - pH 7.0

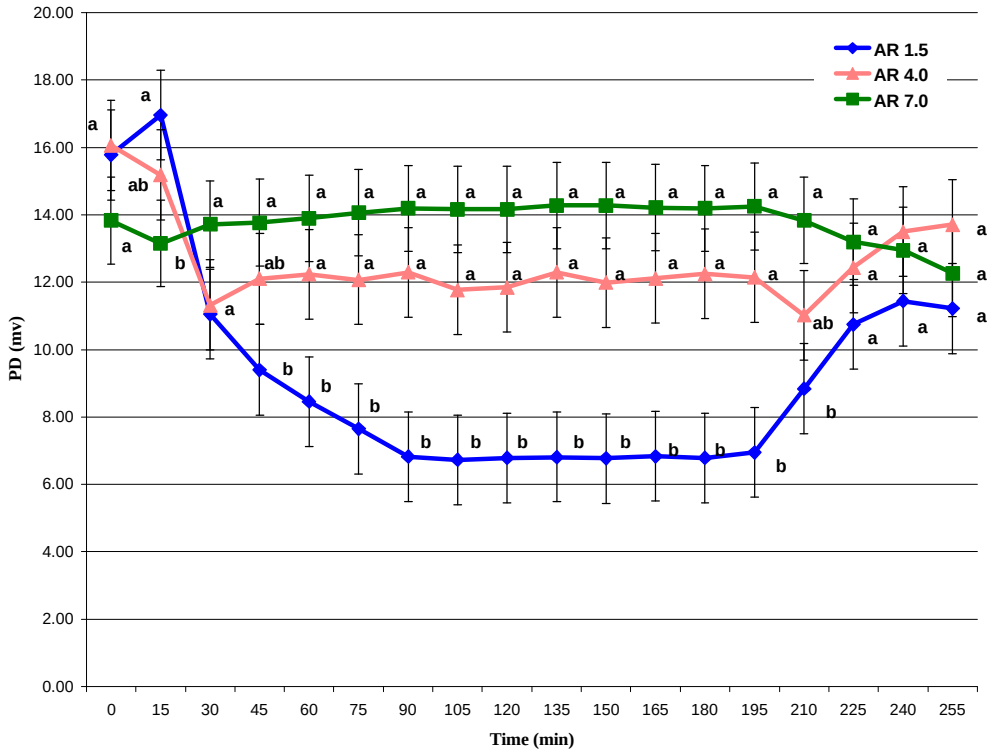


Figure 3-21. Mean PD: Acetic Ringer's Solution

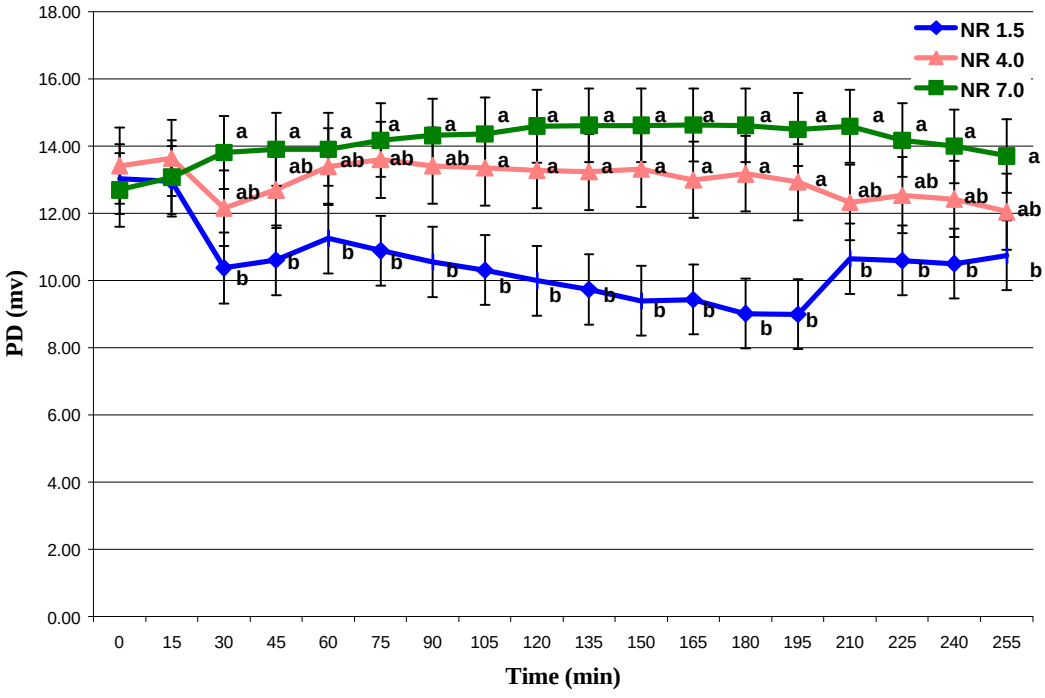


Figure 3-22. Mean PD: Normal Ringer's Solution

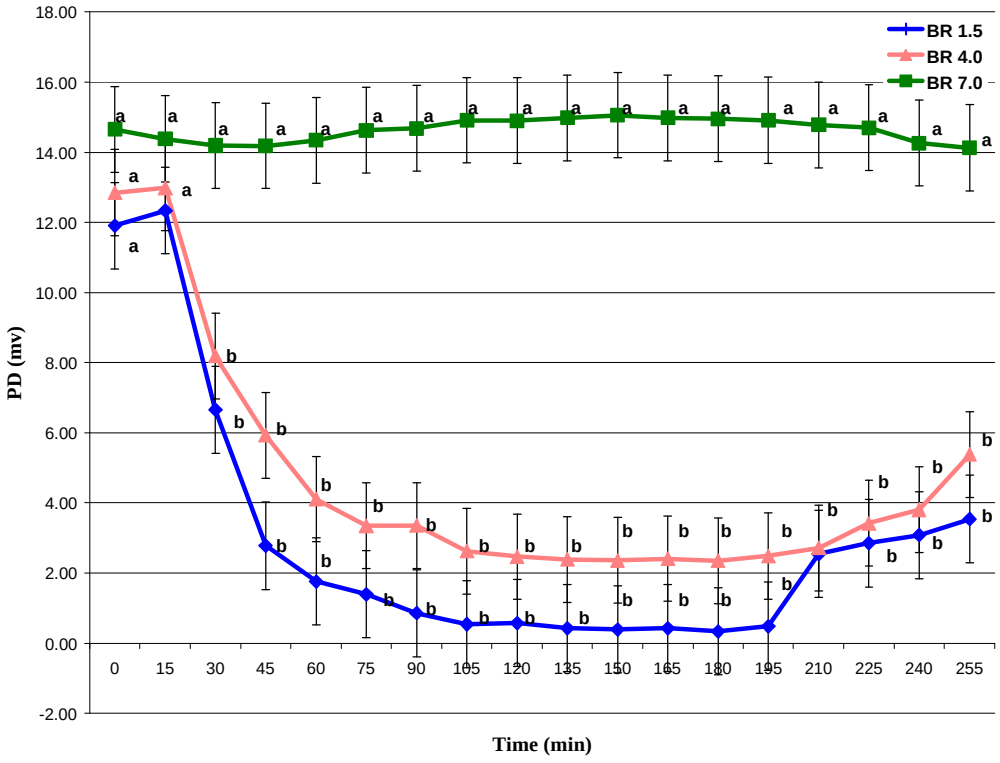


Figure 3-23. Mean PD: Butyric Ringer's Solution

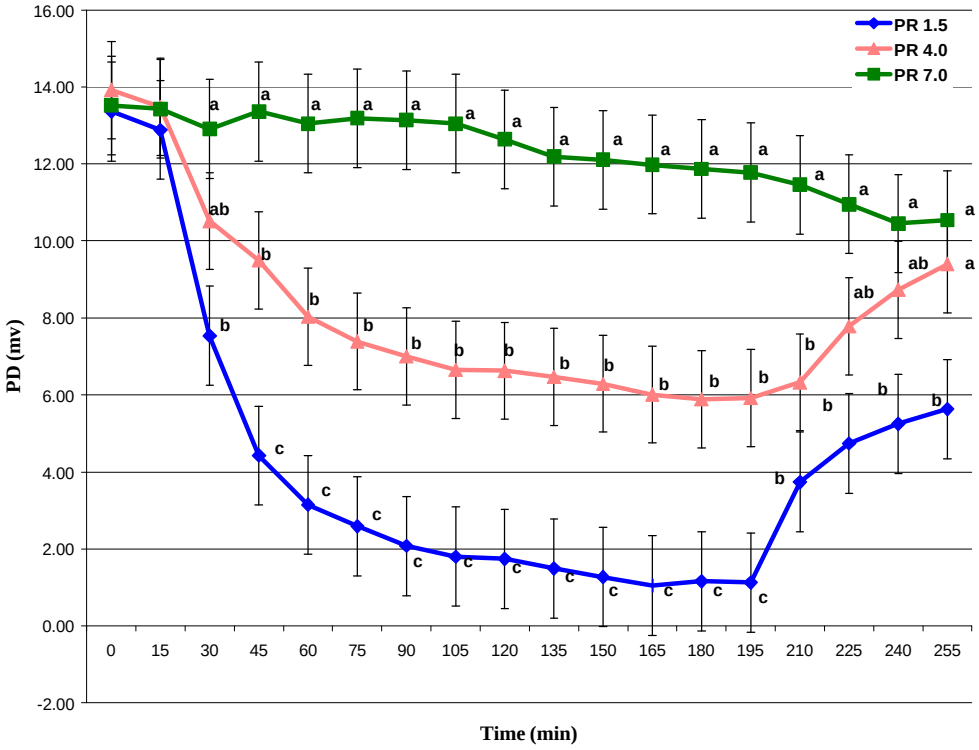


Figure 3-24. Mean PD: Propionic Ringer's Solution

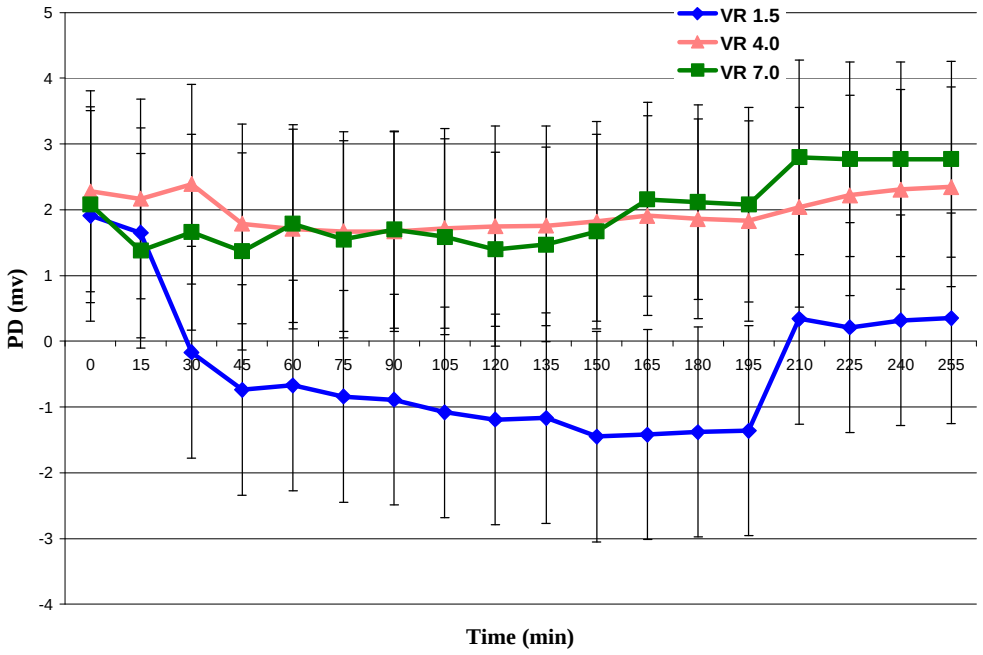


Figure 3-25. Mean PD: Valeric Ringer's Solution

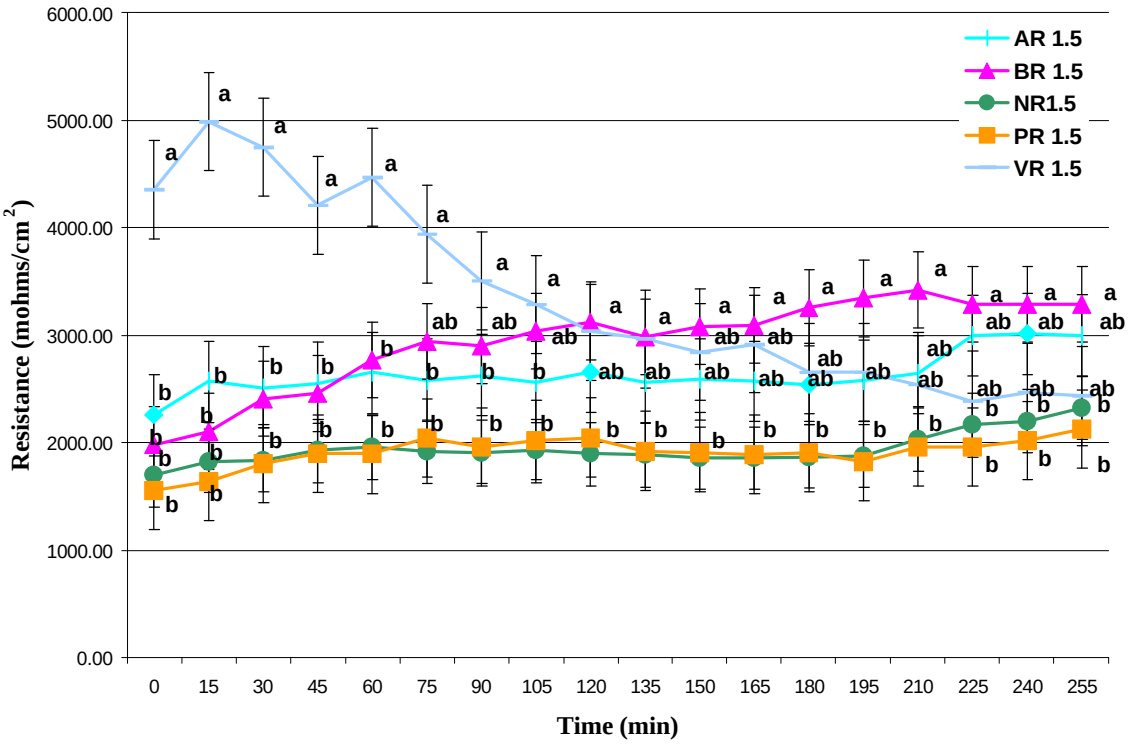


Figure 3-26. Mean Resistance (Comparisons with VR) - pH 1.5

Table 3-5. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	2255.06 ± 377.84^{bg}	2043.88 ± 377.84^{bg}	1687.46 ± 364.03^{bg}
0	Butyric (n=29,29,29)	1975.59 ± 351.64^{bg}	1649.87 ± 346.64^{bg}	1791.12 ± 346.63^{bg}
0	Normal (n=40,34,36)	1697.16 ± 294.42^{bg}	1939.01 ± 321.46^{bg}	1869.13 ± 309.31^{bg}
0	Propionic (n=28,29,27)	1553.49 ± 363.22^{bg}	1777.53 ± 356.91^{bg}	1606.59 ± 364.03^{bg}
0	Valeric (n=16,18,18)	4355.05 ± 456.50^{ag}	4435.99 ± 435.39^{ag}	4027.60 ± 423.64^{ag}
15	Acetic (n=26,25,28)	2565.38 ± 376.94^{b}	2132.73 ± 377.84^{b}	1729.57 ± 363.22^{b}
15	Butyric (n=30,31,29)	2100.13 ± 350.91^{b}	1799.38 ± 345.20^{b}	1893.89 ± 346.63^{b}
15	Normal (n=39,31,36)	1826.28 ± 294.90^{b}	1986.25 ± 323.43^{b}	2044.52 ± 309.31^{b}
15	Propionic (n=28,29,28)	1634.03 ± 363.22^{b}	1820.82 ± 356.91^{b}	1730.65 ± 363.22^{b}
15	Valeric (n=17,17,17)	4986.04 ± 454.65^{a}	4655.06 ± 432.75^{a}	4355.63 ± 425.39^{a}
30	Acetic (n=26,25,28)	2510.65 ± 376.94^{b}	2330.62 ± 377.84^{b}	1861.21 ± 363.22^{b}
30	Butyric (n=29,31,30)	2408.95 ± 351.64^{b}	2100.20 ± 345.20^{b}	2036.08 ± 345.89^{b}
30	Normal (n=38,35,38)	1835.13 ± 295.41^{b}	2130.63 ± 320.88^{b}	2161.60 ± 308.26^{b}
30	Propionic (n=27,29,27)	1805.57 ± 364.03^{b}	2072.59 ± 356.91^{b}	1798.23 ± 364.03^{b}
30	Valeric (n=17,17,17)	4748.25 ± 454.63^{a}	5047.60 ± 437.77^{a}	5116.47 ± 425.40^{a}
45	Acetic (n=25,25,27)	2551.99 ± 377.84^{b}	2566.63 ± 377.84^{b}	1917.25 ± 364.03^{b}
45	Butyric (n=26,30,30)	2453.15 ± 354.16^{b}	2365.33 ± 345.91^{b}	2087.75 ± 345.89^{b}
45	Normal (n=40,34,39)	1926.24 ± 294.42^{b}	2197.09 ± 321.47^{b}	2138.62 ± 307.77^{b}
45	Propionic (n=27,29,28)	1893.89 ± 364.06^{b}	2370.45 ± 356.91^{b}	1849.65 ± 363.22^{b}
45	Valeric (n=16,16,16)	4211.23 ± 456.54^{a}	5058.76 ± 433.84^{a}	4201.40 ± 427.36^{a}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-5 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
60	Acetic (n=26,26,28)	2650.84 ± 376.94^{b}	2687.50 ± 376.94^{b}	1986.86 ± 363.22^{b}
60	Butyric (n=29,29,31)	2765.75 ± 351.64^{b}	2465.62 ± 346.69^{b}	2140.15 ± 345.20^{b}
60	Normal (n=40,34,37)	1955.26 ± 294.42^{b}	2240.56 ± 321.47^{b}	2321.56 ± 308.77^{b}
60	Propionic (n=26,29,28)	1891.71 ± 364.93^{b}	2363.56 ± 356.91^{b}	1857.82 ± 363.22^{b}
60	Valeric (n=17,17,17)	4469.09 ± 454.68^{a}	5351.75 ± 435.89^{a}	4862.76 ± 425.40^{a}
75	Acetic (n=23,26,28)	2580.28 ± 379.87^{b}	2840.45 ± 376.94^{b}	2041.62 ± 363.22^{b}
75	Butyric (n=29,29,31)	2941.12 ± 351.63^{ab}	2672.46 ± 346.72^{b}	2169.32 ± 345.20^{b}
75	Normal (n=43,35,39)	1913.45 ± 293.10^{b}	2314.52 ± 320.89^{b}	2386.20 ± 307.77^{b}
75	Propionic (n=27,29,28)	2038.07 ± 364.05^{b}	2409.26 ± 356.91^{b}	1901.03 ± 363.22^{b}
75	Valeric (n=17,17,17)	3939.36 ± 456.47^{ad}	5221.72 ± 435.89^{ae}	4513.20 ± 425.40^{ade}
90	Acetic (n=26,26,28)	2623.72 ± 376.94^{b}	3016.82 ± 376.94^{b}	2086.73 ± 363.22^{b}
90	Butyric (n=29,26,31)	2900.60 ± 351.63^{ab}	3005.46 ± 349.43^{b}	2217.91 ± 345.20^{b}
90	Normal (n=43,36,39)	1911.35 ± 293.10^{b}	2384.70 ± 320.33^{b}	2398.40 ± 307.77^{b}
90	Propionic (n=26,28,28)	1958.29 ± 364.96^{b}	2591.37 ± 357.75^{b}	1922.74 ± 363.22^{b}
90	Valeric (n=14,18,18)	3503.40 ± 456.54^{ad}	5100.98 ± 442.34^{ae}	5003.10 ± 423.68^{ae}
105	Acetic (n=26,26,28)	2564.02 ± 376.94^{b}	2978.32 ± 376.94^{b}	2125.85 ± 363.22^{b}
105	Butyric (n=29,25,31)	3038.29 ± 351.63^{ab}	2926.28 ± 350.43^{b}	2253.19 ± 345.20^{b}
105	Normal (n=43,36,39)	1922.53 ± 293.10^{b}	2374.87 ± 320.33^{b}	2446.09 ± 307.77^{b}
105	Propionic (n=25,26,27)	2024.51 ± 365.92^{b}	2679.77 ± 359.45^{b}	1959.09 ± 364.03^{b}
105	Valeric (n=15,15,19)	3288.86 ± 454.69^{ad}	5365.25 ± 440.06^{ae}	4826.41 ± 422.12^{ae}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH

^{gh}value differs from initial value after change to NR solution

Table 3-5 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
120	Acetic (n=24,26,28)	2654.13 ± 378.83^{ab}	3062.32 ± 376.94^{b}	2145.15 ± 363.22^{b}
120	Butyric (n=29,23,30)	3115.60 ± 351.66^{ade}	3290.04 ± 352.65^{bd}	2299.05 ± 345.91^{be}
120	Normal (n=43,36,39)	1893.56 ± 293.10^{b}	2426.90 ± 320.33^{b}	2470.69 ± 307.77^{b}
120	Propionic (n=23,26,27)	2042.51 ± 368.03^{b}	2725.26 ± 359.48^{b}	1973.80 ± 364.08^{b}
120	Valeric (n=14,14,21)	3033.00 ± 456.54^{ad}	5148.93 ± 442.46^{ae}	4599.33 ± 419.41^{ae}
135	Acetic (n=25,26,28)	2563.24 ± 377.85^{abde}	3259.53 ± 376.94^{bd}	2167.69 ± 363.22^{be}
135	Butyric (n=28,22,30)	2981.34 ± 352.44^{a}	3186.50 ± 353.91^{b}	2331.27 ± 345.91^{b}
135	Normal (n=43,36,39)	1882.19 ± 293.10^{b}	2461.49 ± 320.33^{b}	2467.93 ± 307.77^{b}
135	Propionic (n=23,27,27)	1917.34 ± 368.03^{b}	2789.84 ± 358.60^{b}	1984.08 ± 364.08^{b}
135	Valeric (n=15,15,21)	2959.14 ± 456.54^{ad}	5174.04 ± 440.06^{ae}	4265.41 ± 419.41^{ae}
150	Acetic (n=25,26,28)	2588.63 ± 377.85^{ab}	3157.40 ± 376.94^{b}	2178.13 ± 363.22^{b}
150	Butyric (n=27,24,30)	3077.11 ± 353.29^{a}	3073.10 ± 351.49^{b}	2371.92 ± 345.91^{b}
150	Normal (n=43,36,37)	1852.98 ± 293.10^{b}	2482.74 ± 320.33^{b}	2439.64 ± 308.76^{b}
150	Propionic (n=26,26,27)	1911.76 ± 364.97^{b}	2891.39 ± 359.50^{b}	2024.76 ± 364.08^{b}
150	Valeric (n=15,15,21)	2843.46 ± 454.69^{abd}	5169.41 ± 440.06^{ae}	4392.81 ± 419.41^{ae}
165	Acetic (n=26,26,28)	2570.23 ± 376.94^{ab}	3222.30 ± 376.94^{b}	2209.45 ± 363.22^{b}
165	Butyric (n=26,23,30)	3086.76 ± 354.18^{a}	3154.85 ± 352.67^{b}	2400.19 ± 345.91^{b}
165	Normal (n=43,36,39)	1856.80 ± 293.10^{b}	2522.97 ± 320.33^{b}	2472.92 ± 307.77^{b}
165	Propionic (n=25,27,27)	1888.84 ± 365.92^{bd}	2934.43 ± 358.60^{be}	2053.23 ± 364.08^{bde}
165	Valeric (n=15,15,21)	2916.41 ± 454.69^{abd}	5133.03 ± 440.06^{ae}	4595.43 ± 419.41^{ae}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH

^{gh}value differs from initial value after change to NR solution

Table 3-5 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mohms/cm^2) ±SEM @ pH 1.5	Mean (mohms/cm^2) ±SEM @ pH 4.0	Mean (mohms/cm^2) ±SEM @ pH 7.0
180	Acetic (n=25,26,28)	2539.51 ± 377.85^{ab}	3232.47 ± 376.94^{b}	2214.27 ± 363.22^{b}
180	Butyric (n=28,23,30)	3256.77 ± 352.45^{a}	3194.38 ± 352.67^{b}	2431.17 ± 345.91^{b}
180	Normal (n=43,36,39)	1870.43 ± 293.10^{b}	2592.17 ± 320.33^{b}	2522.04 ± 307.77^{b}
180	Propionic (n=25,27,27)	1906.20 ± 365.90^{bd}	2946.93 ± 358.58^{be}	2093.83 ± 364.08^{bde}
180	Valeric (n=15,15,21)	2649.00 ± 454.69^{abd}	5078.91 ± 440.06^{ae}	4494.16 ± 419.41^{ae}
195	Acetic (n=25,24,26)	2579.75 ± 377.84^{ab}	3248.81 ± 378.81^{b}	2230.61 ± 364.90^{b}
195	Butyric (n=24,22,27)	3342.57 ± 356.19^{a}	3222.03 ± 353.91^{b}	2461.07 ± 348.27^{b}
195	Normal (n=41,35,38)	1874.86 ± 293.96^{b}	2586.97 ± 320.88^{b}	2530.94 ± 308.25^{b}
195	Propionic (n=24,26,26)	1828.58 ± 366.90^{bd}	3018.77 ± 359.50^{be}	2114.09 ± 364.94^{bde}
195	Valeric (n=16,16,21)	2650.28 ± 454.69^{abd}	5000.64 ± 437.80^{ae}	4219.95 ± 419.41^{ae}
210	Acetic (n=26,26,28)	2647.27 ± 376.94^{ab}	3234.70 ± 376.94^{b}	2321.62 ± 363.22^{b}
210	Butyric (n=27,24,30)	3417.19 ± 353.26^{a}	2946.53 ± 351.48^{b}	2433.52 ± 345.89^{b}
210	Normal (n=42,35,38)	2028.07 ± 293.52^{b}	2481.30 ± 320.89^{b}	2552.85 ± 308.26^{b}
210	Propionic (n=25,25,26)	1963.35 ± 365.86^{b}	2876.10 ± 360.46^{b}	2074.64 ± 364.94^{b}
210	Valeric (n=13,13,16)	2538.76 ± 456.46^{abd}	5037.81 ± 445.18^{ae}	3915.88 ± 427.33^{ae}
225	Acetic (n=26,26,28)	2993.81 ± 376.94^{abde}	3232.53 ± 376.94^{abd}	2173.75 ± 363.22^{be}
225	Butyric (n=27,29,31)	3285.72 ± 353.27^{a}	2704.45 ± 346.77^{b}	2445.42 ± 345.20^{b}
225	Normal (n=43,35,38)	2161.68 ± 293.10^{b}	2505.54 ± 320.89^{b}	2622.53 ± 308.26^{b}
225	Propionic (n=26,25,27)	1957.63 ± 364.90^{b}	2921.46 ± 360.46^{b}	2078.59 ± 364.08^{b}
225	Valeric (n=16,16,17)	2388.15 ± 458.50^{abd}	4307.89 ± 437.76^{ae}	3033.26 ± 425.41^{ad}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH

^{gh}value differs from initial value after change to NR solution

Table 3-5 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on resistance in exposed equine non-glandular mucosa.

Time	**VFA Solution**	**Mean (mohms/cm^2) ±SEM @ pH 1.5**	**Mean (mohms/cm^2) ±SEM @ pH 4.0**	**Mean (mohms/cm^2) ±SEM @ pH 7.0**
240	Acetic (n=25,26,28)	3010.62 ± 377.84^{ab}	3196.33 ± 376.94^{b}	2245.40 ± 363.22^{b}
240	Butyric (n=29,29,31)	3286.57 ± 351.64^{a}	2788.61 ± 346.77^{b}	2481.85 ± 345.20^{b}
240	Normal (n=43,35,38)	2201.37 ± 293.10^{b}	2534.28 ± 320.89^{b}	2655.46 ± 308.26^{b}
240	Propionic (n=27,25,27)	2021.48 ± 364.03^{b}	2921.51 ± 360.46^{b}	2110.49 ± 364.08^{b}
240	Valeric (n=14,14,16)	2465.64 ± 456.46^{abd}	4407.36 ± 442.30^{ae}	2970.65 ± 427.33^{ad}
255	Acetic (n=24,24,26)	2999.18 ± 378.82^{abh}	2927.77 ± 378.81^{bh}	2185.53 ± 364.90^{bh}
255	Butyric (n=26,27,29)	3282.73 ± 354.16^{ah}	2820.18 ± 348.47^{bh}	2523.20 ± 346.63^{bh}
255	Normal (n=42,34,37)	2319.73 ± 293.52^{bh}	2560.05 ± 321.47^{bh}	2671.21 ± 308.77^{bh}
255	Propionic (n=27,24,27)	2124.30 ± 364.03^{bh}	2903.67 ± 361.46^{bh}	2145.78 ± 364.08^{bh}
255	Valeric (n=14,14,16)	2434.88 ± 460.85^{abdh}	4243.96 ± 442.30^{aeh}	2952.32 ± 427.34^{adh}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH

^{gh}value differs from initial value after change to NR solution

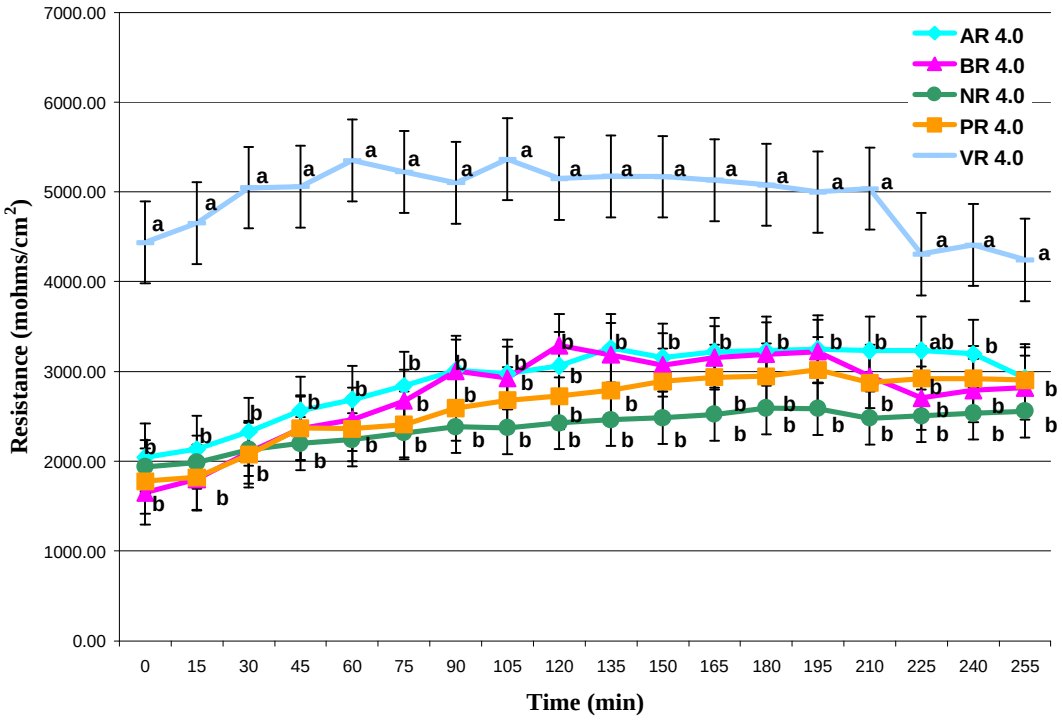


Figure 3-27. Mean Resistance (Comparisons with VR) - pH 4.0

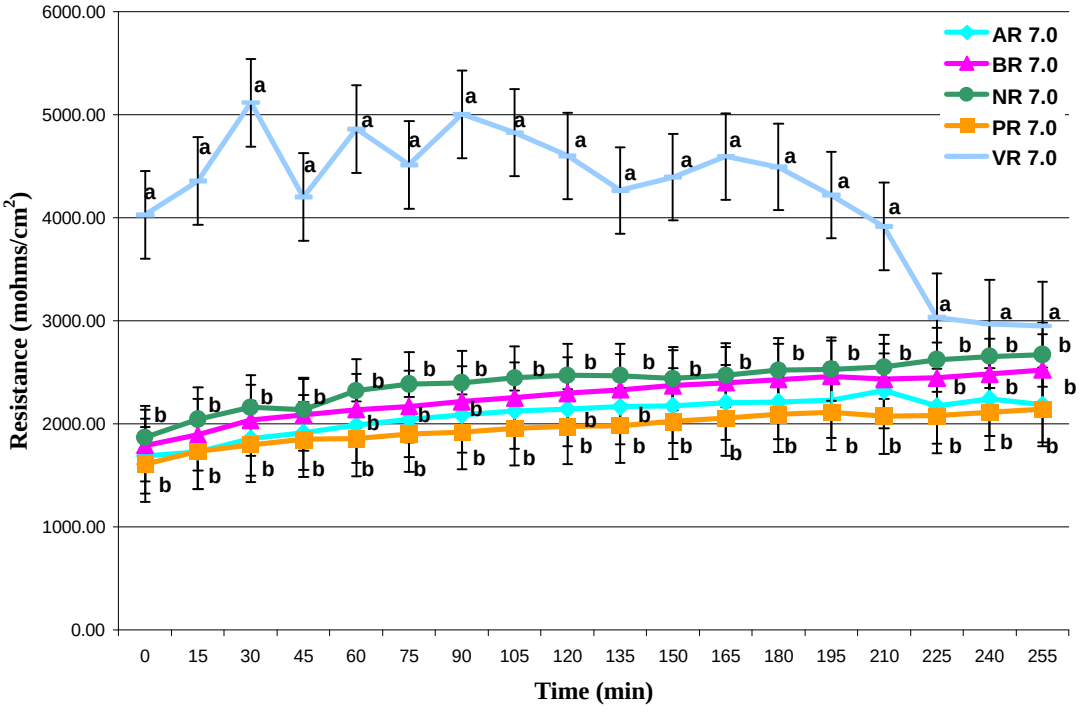


Figure 3-28. Mean Resistance (Comparisons with VR) - pH 7.0

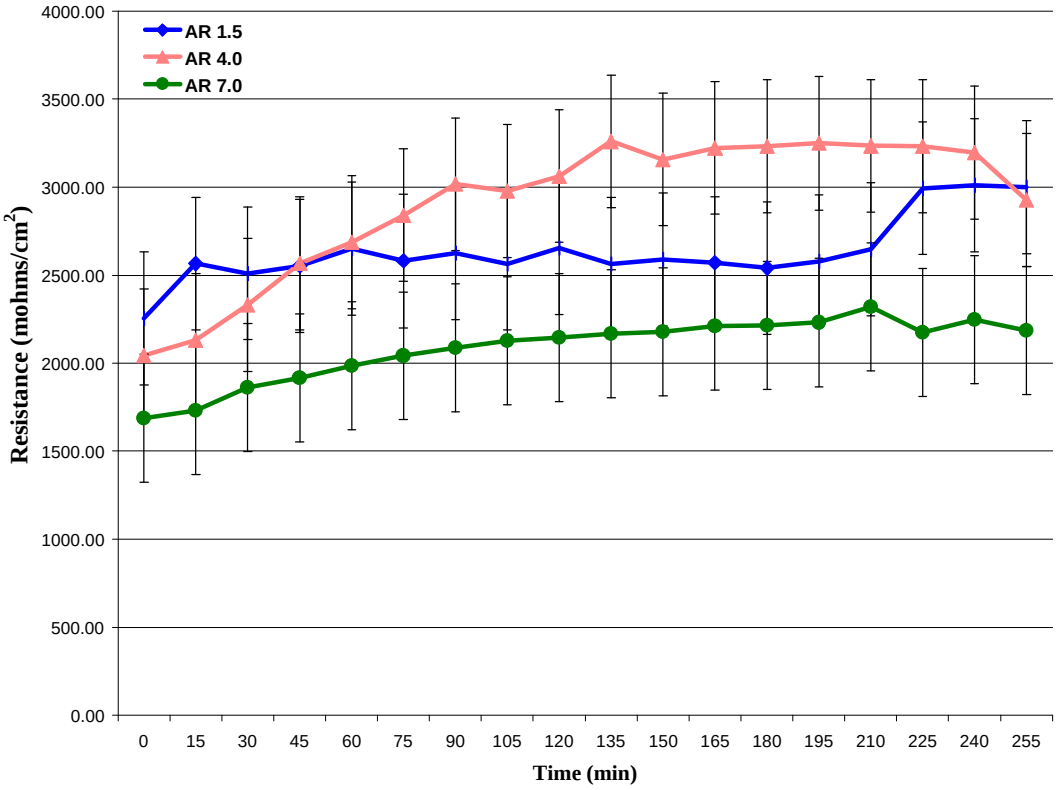


Figure 3-29. Mean Resistance: Acetic Ringer's Solution

compared to pH 4.0 or 7.0 (Figure 3-30). There was a trend for normal Ringer's solution to decrease R at pH 1.5 and 4.0 compared to pH 7.0 (Figure 3-31). Propionic acid decreased R ($P<0.01$) at pH 1.5 and 7.0 compared to pH 4.0 (Figure 3-32). Valeric acid increased R at pH 1.5 and 4.0 and decreased ($P<0.01$) over time (Figure 3-33).

Propionic acid decreased ($P<0.01$) G following the 30 min acclimation period at pH 1.5. Valeric acid also decreased ($P<0.01$) G following the 30 min acclimation period at pH 1.5 (Figure 3-34, Table 3-6). There were no significant differences in G at pH 4.0 (Figure 3-35, Table 3-6). Valeric acid decreased ($P<0.01$) G following the 30 min acclimation period at pH 7.0 (Figure 3-36, Table 3-6).

Acetic acid decreased ($P<0.01$) G at pH 1.5 and 4.0 (Figure 3-37). There was a trend for butyric acid to decrease G at pH 4 and 7.0 compared to pH 1.5 (Figure 3-38). Normal Ringer's solution decreased ($P<0.01$) G at pH 7.0 followed by pH 4.0 (Figure 3-39). There was a trend for propionic acid to decrease G at pH 4.0 compared to pH 1.5 and 7.0 (Figure 3-40). Valeric acid increased ($P<0.01$) G at pH 1.5 compared to pH 4.0 or 7.0 (Figure 3-41).

Homogenized tissues were analyzed by gas chromatography. Significant differences were found only when tissues were exposed to PRS or BRS. When tissues were exposed to PRS, propionic acid absorption was higher ($P<0.01$) compared to exposure to other VFAs or normal Ringer's solution (Table 3-7). When tissues were exposed to butyric acid, butyric acid absorption was higher ($P<0.01$) compared to exposure to other VFAs or normal Ringer's solution (Table 3-8). Butyric acid absorption was also higher ($P<0.01$) at pH 1.5 and 4.0 compared to pH 7.0 (Table 3-9). Butyric acid absorption was highest ($P<0.01$) in tissues exposed to butyric acid at pH 4.0 and butyric acid at pH 1.5 followed

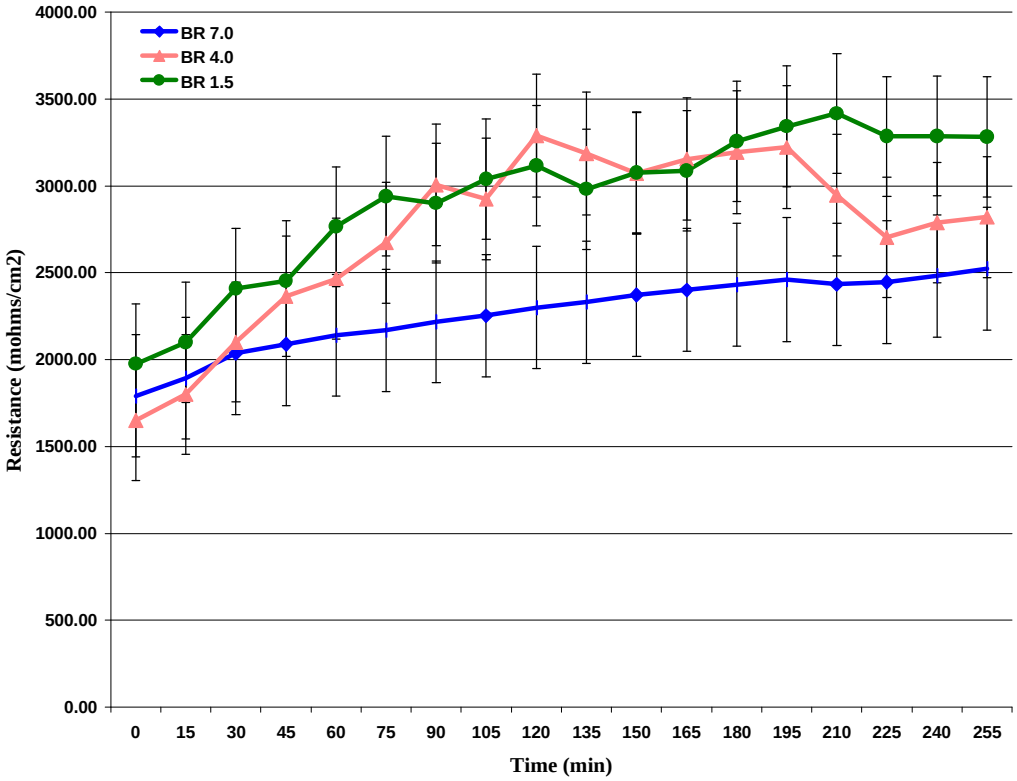


Figure 3-30. Mean Resistance: Butyric Ringer's Solution

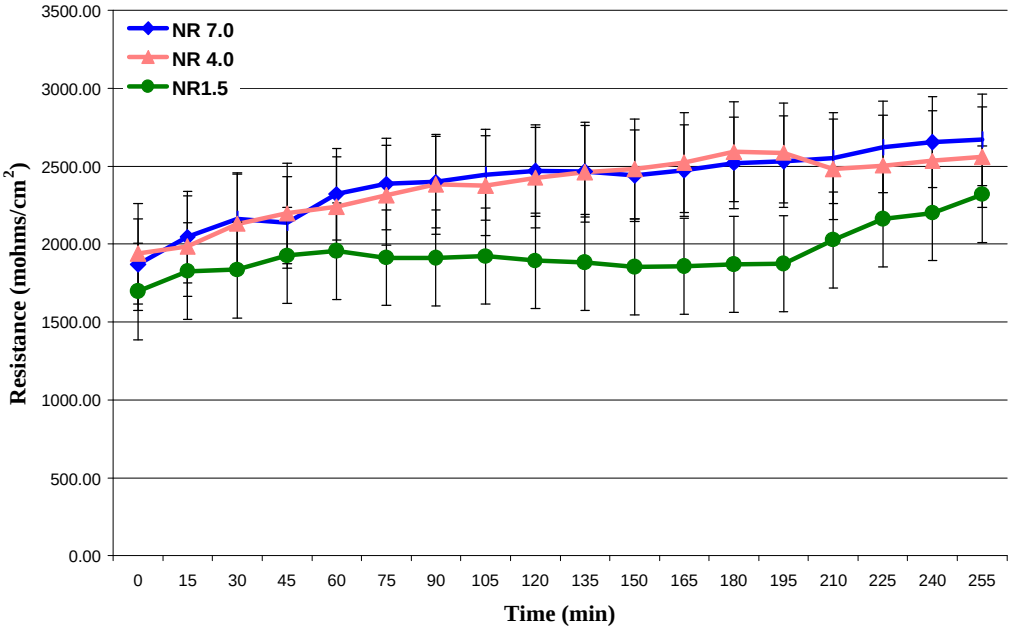


Figure 3-31. Mean Resistance: Normal Ringer's Solution

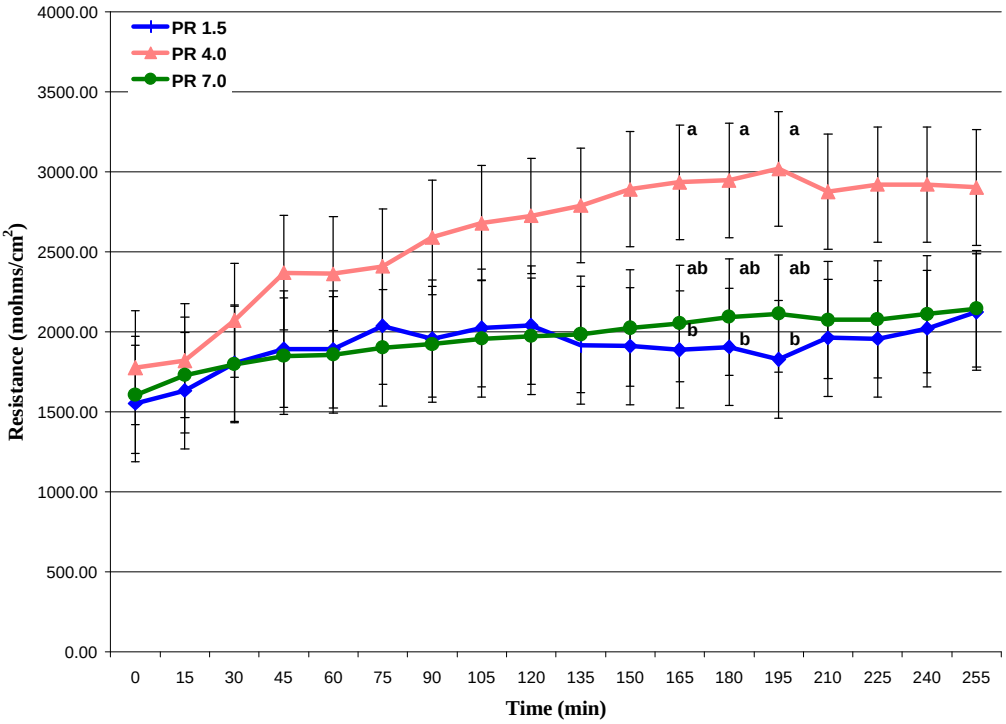


Figure 3-32. Mean Resistance: Propionic Ringer's Solution

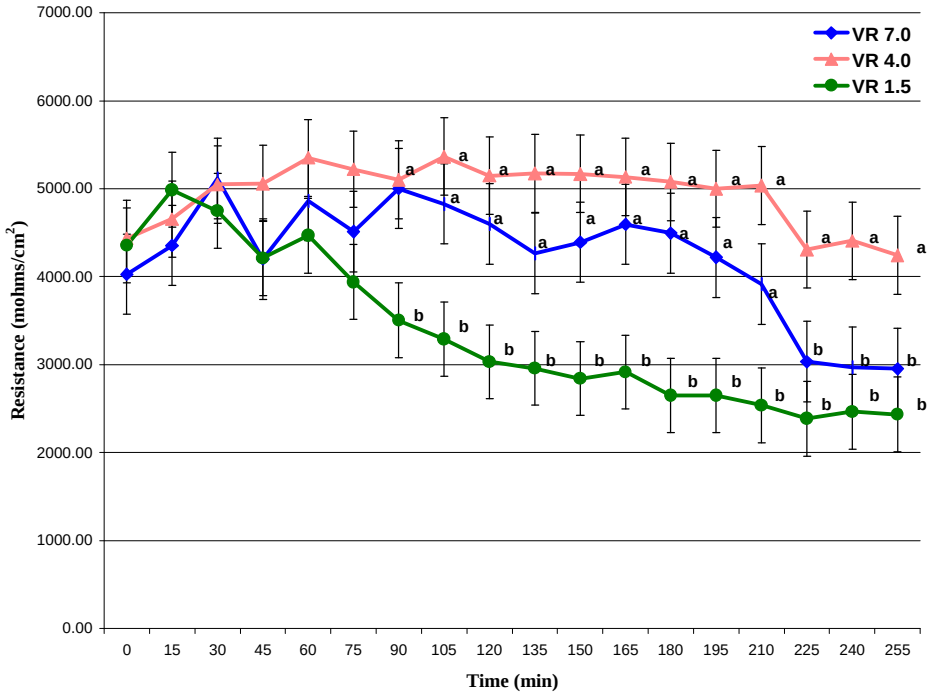


Figure 3-33. Mean Resistance: Valeric Ringer's Solution

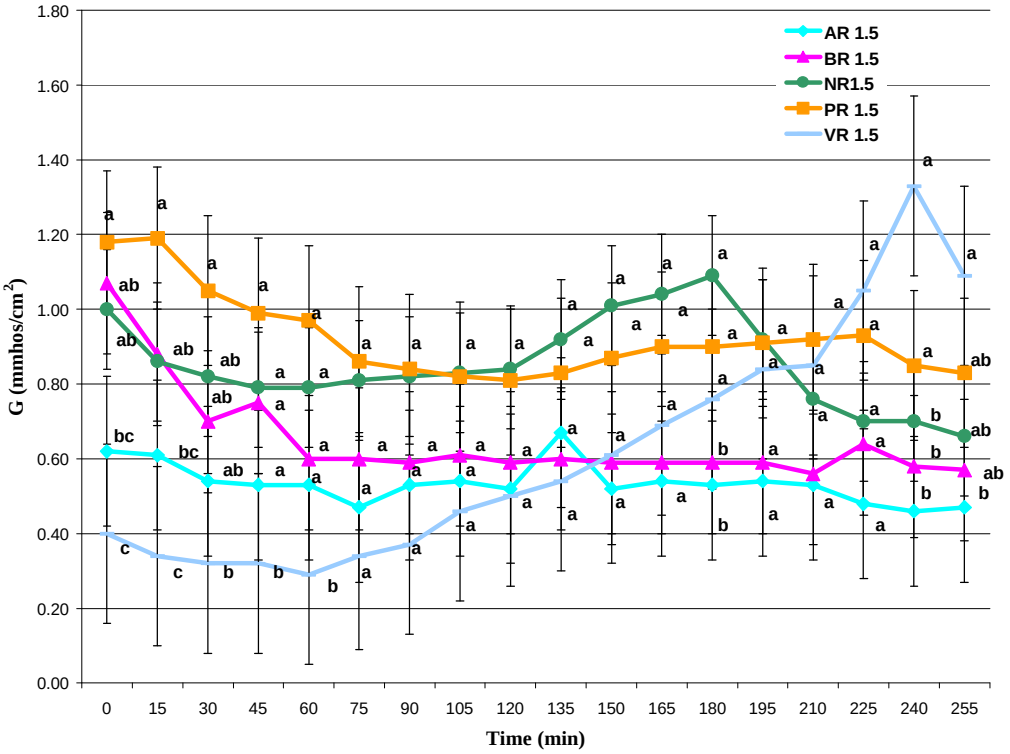


Figure 3-34. Mean Conductance (Comparisons with VR) - pH 1.5

Table 3-6. Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mmhos/cm^2) ±SEM @ pH 1.5	Mean (mmhos/cm^2) ±SEM @ pH 4.0	Mean (mmhos/cm^2) ±SEM @ pH 7.0
0	Acetic (n=25,25,27)	0.62 ± 0.20^{bcdg}	0.71 ± 0.20^{abdg}	1.41 ± 0.20^{aeg}
0	Butyric (n=29,29,29)	1.07 ± 0.19^{bcg}	1.23 ± 0.19^{ag}	1.19 ± 0.19^{ag}
0	Normal (n=40,34,36)	1.00 ± 0.16^{abdg}	1.15 ± 0.17^{adg}	1.47 ± 0.17^{aeg}
0	Propionic (n=28,29,27)	1.18 ± 0.19^{ag}	1.21 ± 0.19^{ag}	1.16 ± 0.20^{ag}
0	Valeric (n=18,18,19)	0.40 ± 0.24^{cg}	0.55 ± 0.23^{bg}	0.59 ± 0.23^{bg}
15	Acetic (n=26,25,28)	0.61 ± 0.20^{abd}	0.68 ± 0.20^{abd}	1.26 ± 0.19^{ae}
15	Butyric (n=30,31,29)	0.88 ± 0.19^{bc}	1.14 ± 0.19^{a}	1.05 ± 0.19^{a}
15	Normal (n=40,32,36)	0.86 ± 0.16^{abd}	1.04 ± 0.17^{abd}	1.34 ± 0.17^{ae}
15	Propionic (n=28,29,28)	1.19 ± 0.19^{a}	1.14 ± 0.19^{a}	0.99 ± 0.19^{a}
15	Valeric (n=18,18,19)	0.34 ± 0.24^{c}	0.51 ± 0.23^{b}	0.51 ± 0.23^{b}
30	Acetic (n=26,25,28)	0.54 ± 0.20^{abd}	0.60 ± 0.20^{d}	1.14 ± 0.19^{abe}
30	Butyric (n=29,31,30)	0.70 ± 0.19^{ab}	0.94 ± 0.19	0.99 ± 0.19^{bc}
30	Normal (n=38,35,38)	0.82 ± 0.16^{abd}	0.92 ± 0.17^{d}	1.57 ± 0.17^{ae}
30	Propionic (n=27,29,27)	1.05 ± 0.20^{a}	0.98 ± 0.19	0.90 ± 0.20^{bc}
30	Valeric (n=17,16,19)	0.32 ± 0.24^{c}	0.51 ± 0.23	0.45 ± 0.23^{c}
45	Acetic (n=25,25,27)	0.53 ± 0.20^{a}	0.55 ± 0.20	1.08 ± 0.20^{a}
45	Butyric (n=26,30,30)	0.75 ± 0.19^{a}	0.83 ± 0.19	1.00 ± 0.19^{a}
45	Normal (n=40,34,39)	0.79 ± 0.16^{ad}	0.88 ± 0.17^{d}	1.37 ± 0.17^{ae}
45	Propionic (n=27,29,28)	0.99 ± 0.20^{a}	0.86 ± 0.19	0.90 ± 0.19^{a}
45	Valeric (n=18,20,17)	0.32 ± 0.24^{b}	0.63 ± 0.23	0.46 ± 0.23^{b}
60	Acetic (n=26,26,28)	0.53 ± 0.20^{a}	0.51 ± 0.20	1.03 ± 0.19^{a}
60	Butyric (n=29,31,31)	0.60 ± 0.19^{a}	0.75 ± 0.19	0.96 ± 0.19^{a}
60	Normal (n=40,34,37)	0.79 ± 0.16^{ad}	0.89 ± 0.17^{de}	1.26 ± 0.17^{ae}
60	Propionic (n=27,29,28)	0.97 ± 0.20^{a}	0.83 ± 0.19	0.94 ± 0.19^{a}
60	Valeric (n=17,17,18)	0.29 ± 0.24^{b}	0.47 ± 0.23	0.46 ± 0.23^{b}
75	Acetic (n=25,26,28)	0.47 ± 0.20^{a}	0.48 ± 0.20	0.98 ± 0.19^{a}
75	Butyric (n=29,31,31)	0.60 ± 0.19^{a}	0.67 ± 0.19	0.93 ± 0.19^{a}
75	Normal (n=43,35,39)	0.81 ± 0.16^{a}	0.86 ± 0.17	1.19 ± 0.17^{a}
75	Propionic (n=27,29,28)	0.86 ± 0.20^{a}	0.78 ± 0.19	0.87 ± 0.19^{a}
75	Valeric (n=16,17,18)	0.34 ± 0.24^{a}	0.46 ± 0.23	0.45 ± 0.23^{b}

SEM=standard error of the mean

Means not sharing like letters differ (P≤0.01); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

Table 3-6 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mmhos/cm^2) ±SEM @ pH 1.5	Mean (mmhos/cm^2) ±SEM @ pH 4.0	Mean (mmhos/cm^2) ±SEM @ pH 7.0
90	Acetic (n=26,26,28)	0.53 ± 0.20[a]	0.45 ± 0.20	0.94 ± 0.19[a]
90	Butyric (n=30,29,31)	0.59 ± 0.19[a]	0.61 ± 0.19	0.91 ± 0.19[a]
90	Normal (n=43,36,39)	0.82 ± 0.16[a]	0.83 ± 0.17	1.18 ± 0.17[a]
90	Propionic (n=27,29,28)	0.84 ± 0.20[a]	0.74 ± 0.19	0.87 ± 0.109[a]
90	Valeric (n=18,18,19)	0.37 ± 0.24[a]	0.46 ± 0.23	0.46 ± 0.23[b]
105	Acetic (n=26,26,28)	0.54 ± 0.20[a]	0.46 ± 0.20	0.91 ± 0.19[a]
105	Butyric (n=29,27,31)	0.61 ± 0.19[a]	0.66 ± 0.19	0.88 ± 0.19[a]
105	Normal (n=43,36,39)	0.83 ± 0.16[a]	0.82 ± 0.17	1.11 ± 0.17[a]
105	Propionic (n=26,27,27)	0.82 ± 0.20[a]	0.69 ± 0.19	0.83 ± 0.20[a]
105	Valeric (n=18,20,20)	0.46 ± 0.24[a]	0.51 ± 0.23	0.45 ± 0.23[b]
120	Acetic (n=24,26,28)	0.52 ± 0.20[a]	0.44 ± 0.20	0.88 ± 0.19[a]
120	Butyric (n=29,27,30)	0.59 ± 0.19[a]	0.60 ± 0.19	0.83 ± 0.19[a]
120	Normal (n=43,36,39)	0.84 ± 0.16[a]	0.80 ± 0.17	1.07 ± 0.17[a]
120	Propionic (n=25,28,27)	0.81 ± 0.20[a]	0.64 ± 0.19	0.85 ± 0.20[a]
120	Valeric (n=18,19,21)	0.50 ± 0.24[a]	0.43 ± 0.23	0.45 ± 0.23[b]
135	Acetic (n=26,26,28)	0.67 ± 0.20[a]	0.43 ± 0.20	0.86 ± 0.19[a]
135	Butyric (n=28,26,30)	0.60 ± 0.19[a]	0.57 ± 0.19	0.85 ± 0.19[a]
135	Normal (n=43,36,39)	0.92 ± 0.16[a]	0.78 ± 0.17	1.04 ± 0.17[a]
135	Propionic (n=24,29,27)	0.83 ± 0.20[a]	0.61 ± 0.19	0.83 ± 0.20[a]
135	Valeric (n=18,20,21)	0.54 ± 0.24[a]	0.38 ± 0.23	0.42 ± 0.23[b]
150	Acetic (n=26,26,28)	0.52 ± 0.20[a]	0.43 ± 0.20	0.84 ± 0.19[a]
150	Butyric (n=29,27,30)	0.59 ± 0.19[a]	0.62 ± 0.19	0.82 ± 0.19[a]
150	Normal (n=43,36,37)	1.01 ± 0.16[a]	0.79 ± 0.17	1.03 ± 0.17[a]
150	Propionic (n=26,28,27)	0.87 ± 0.20[a]	0.59 ± 0.19	0.83 ± 0.20[a]
150	Valeric (n=18,19,21)	0.61 ± 0.24[a]	0.45 ± 0.23	0.45 ± 0.23[b]
165	Acetic (n=26,26,28)	0.54 ± 0.20[a]	0.42 ± 0.20	0.82 ± 0.19
165	Butyric (n=28,28,30)	0.59 ± 0.19[a]	0.59 ± 0.19	0.81 ± 0.19
165	Normal (n=43,36,39)	1.04 ± 0.16[a]	0.81 ± 0.17	0.95 ± 0.17
165	Propionic (n=26,29,27)	0.90 ± 0.20[a]	0.57 ± 0.19	0.82 ± 0.20
165	Valeric (n=18,19,21)	0.69 ± 0.24[a]	0.39 ± 0.23	0.44 ± 0.23

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); [abc]for differences within treatment, [def]for differences within pH; [gh]value differs from initial value after change to NR solution

Table 3-6 (cont.). Effect of time and VFA Ringer's solution at pH 1.5, 4.0, and 7.0 on conductance in exposed equine non-glandular mucosa.

Time	VFA Solution	Mean (mmhos/cm^2) ±SEM @ pH 1.5	Mean (mmhos/cm^2) ±SEM @ pH 4.0	Mean (mmhos/cm^2) ±SEM @ pH 7.0
180	Acetic (n=26,26,28)	0.53 ± 0.20^{b}	0.42 ± 0.20	0.81 ± 0.19
180	Butyric (n=29,29,30)	0.59 ± 0.19^{b}	0.55 ± 0.19	0.78 ± 0.19
180	Normal (n=43,36,39)	1.09 ± 0.16^{a}	0.75 ± 0.17	0.92 ± 0.17
180	Propionic (n=25,28,27)	0.90 ± 0.20^{a}	0.57 ± 0.19	0.83 ± 0.20
180	Valeric (n=18,19,21)	0.76 ± 0.24^{a}	0.38 ± 0.23	0.44 ± 0.23
195	Acetic (n=25,24,26)	0.54 ± 0.20^{a}	0.42 ± 0.20	0.78 ± 0.20
195	Butyric (n=24,25,27)	0.59 ± 0.19^{a}	0.55 ± 0.19	0.76 ± 0.19
195	Normal (n=41,35,38)	0.92 ± 0.16^{a}	0.74 ± 0.17	0.88 ± 0.17
195	Propionic (n=26,28,26)	0.91 ± 0.20^{a}	0.55 ± 0.19	0.83 ± 0.20
195	Valeric (n=18,18,21)	0.84 ± 0.24^{a}	0.35 ± 0.23	0.41 ± 0.23
210	Acetic (n=26,26,28)	0.53 ± 0.20^{a}	0.42 ± 0.20	0.81 ± 0.20
210	Butyric (n=28,29,30)	0.56 ± 0.19^{a}	0.69 ± 0.19	0.78 ± 0.19
210	Normal (n=43,35,38)	0.76 ± 0.16^{a}	0.78 ± 0.17	0.89 ± 0.17
210	Propionic (n=26,27,26)	0.92 ± 0.20^{a}	0.60 ± 0.19	0.81 ± 0.20
210	Valeric (n=17,20,19)	0.85 ± 0.24^{a}	0.41 ± 0.23	0.45 ± 0.23
225	Acetic (n=26,26,28)	0.48 ± 0.20^{a}	0.42 ± 0.20	0.80 ± 0.19
225	Butyric (n=28,30,31)	0.64 ± 0.19^{a}	0.73 ± 0.19	0.76 ± 0.19
225	Normal (n=43,35,38)	0.70 ± 0.16^{a}	0.78 ± 0.17	0.85 ± 0.17
225	Propionic (n=26,27,27)	0.93 ± 0.20^{a}	0.57 ± 0.19	0.77 ± 0.20
225	Valeric (n=17,20,20)	1.05 ± 0.24^{a}	0.44 ± 0.23	0.51 ± 0.23
240	Acetic (n=25,26,28)	0.46 ± 0.20^{b}	0.43 ± 0.20	0.77 ± 0.19
240	Butyric (n=30,30,31)	0.58 ± 0.19^{b}	0.71 ± 0.19	0.74 ± 0.19
240	Normal (n=43,35,38)	0.70 ± 0.16^{b}	0.78 ± 0.17	0.83 ± 0.17
240	Propionic (n=27,27,27)	0.85 ± 0.20^{a}	0.57 ± 0.19	0.75 ± 0.20
240	Valeric (n=17,17,19)	1.33 ± 0.24^{ad}	0.41 ± 0.23^{e}	0.45 ± 0.23^{e}
255	Acetic (n=24,24,26)	0.47 ± 0.20^{bg}	0.44 ± 0.20^{h}	0.75 ± 0.20^{h}
255	Butyric (n=28,28,29)	0.57 ± 0.19^{bh}	0.67 ± 0.19^{h}	0.72 ± 0.19^{h}
255	Normal (n=42,34,37)	0.66 ± 0.16^{abh}	0.76 ± 0.17^{h}	0.82 ± 0.17^{h}
255	Propionic (n=27,27,27)	0.83 ± 0.20^{abh}	0.59 ± 0.19^{h}	0.74 ± 0.20^{h}
255	Valeric (n=18,16,18)	1.09 ± 0.24^{adh}	0.41 ± 0.24^{eg}	0.53 ± 0.23^{deg}

SEM=standard error of the mean

Means not sharing like letters differ ($P \leq 0.01$); ^{abc}for differences within treatment, ^{def}for differences within pH; ^{gh}value differs from initial value after change to NR solution

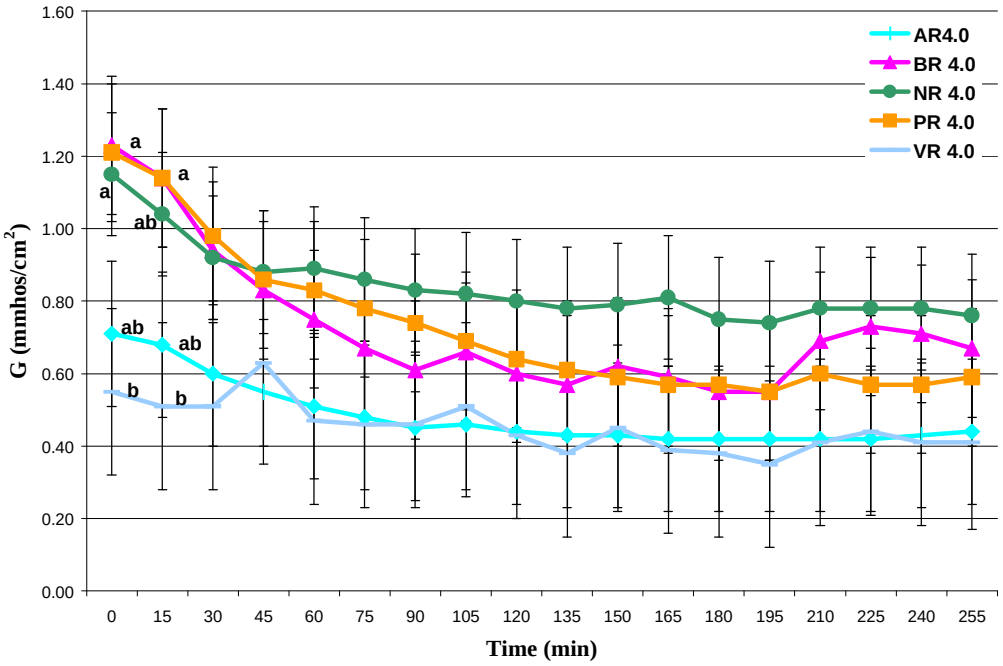


Figure 3-35. Mean Conductance (Comparisons with VR) - pH 4.0

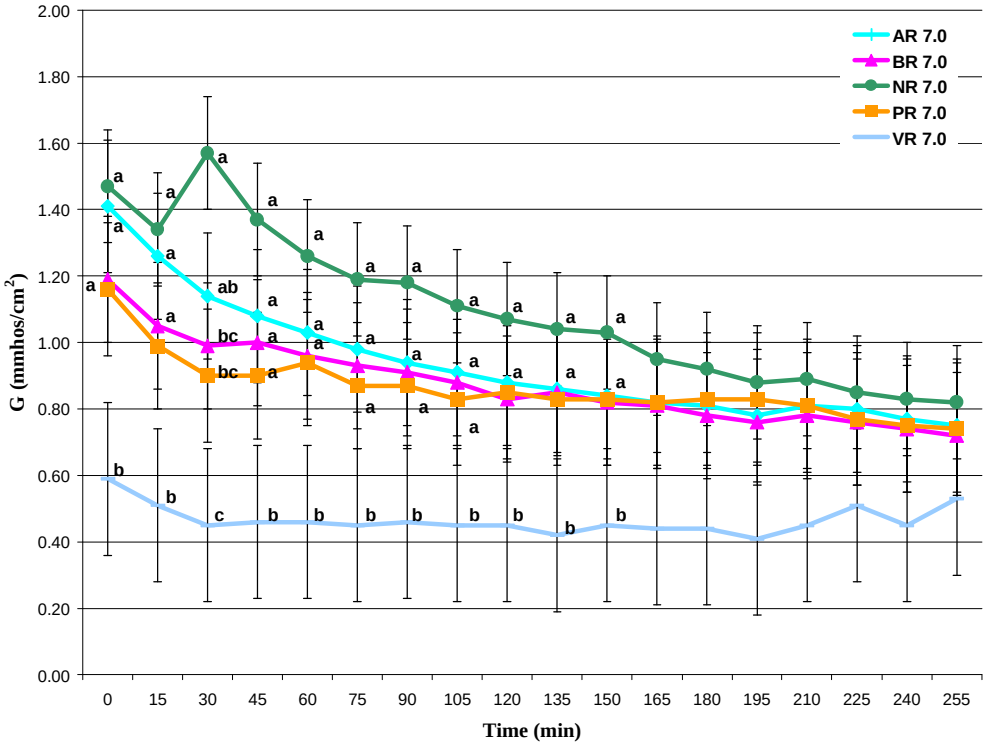


Figure 3-36. Mean Conductance (Comparisons with VR) - pH 7.0

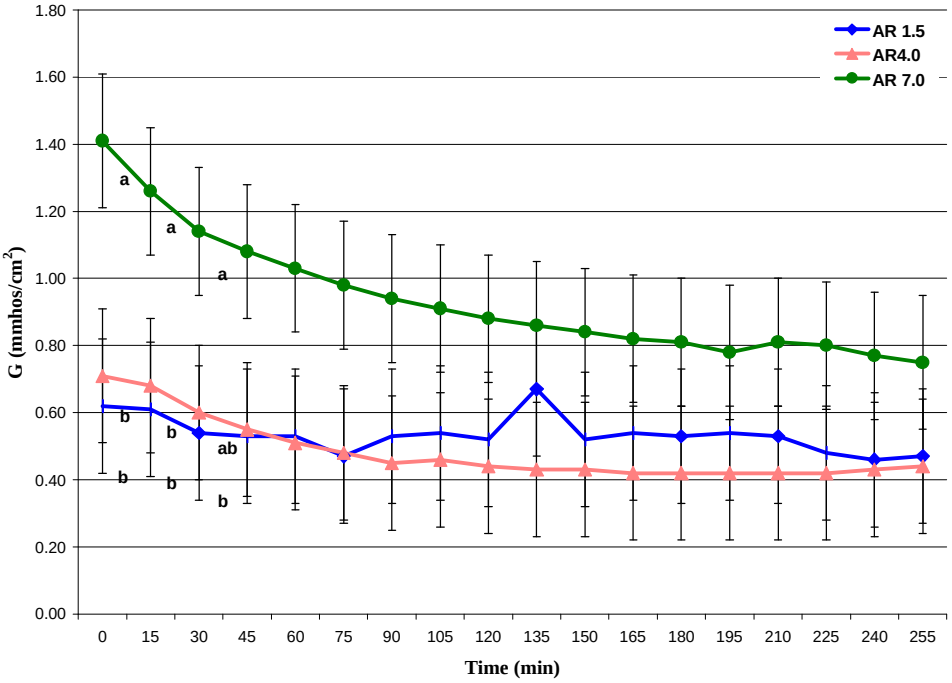


Figure 3-37. Mean Conductance: Acetic Ringer's Solution

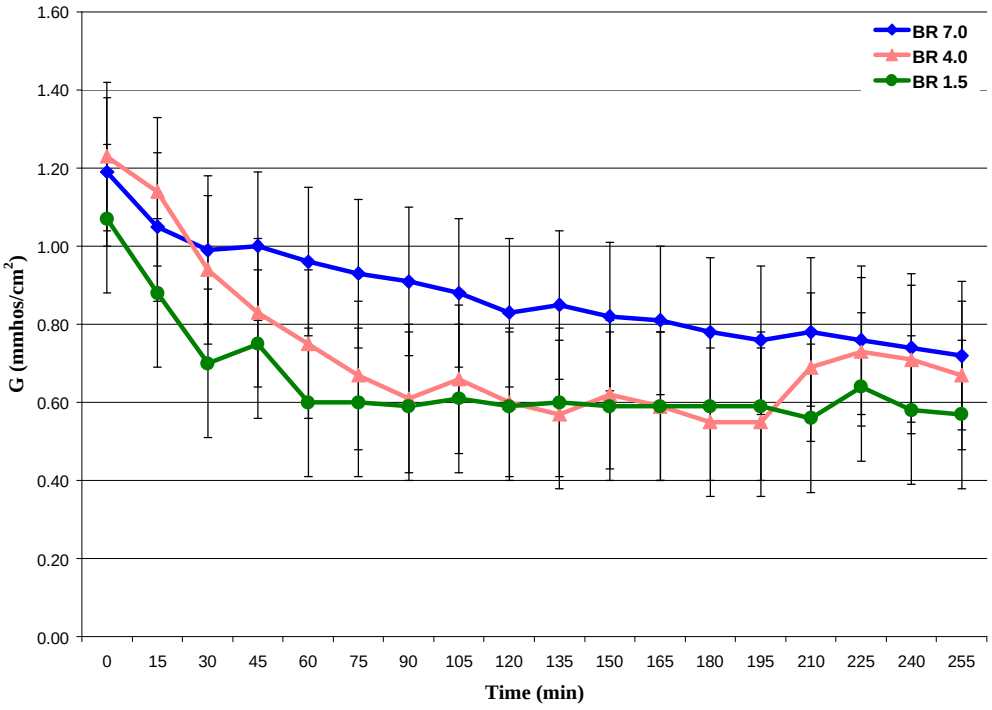


Figure 3-38. Mean Conductance: Butyric Ringer's Solution

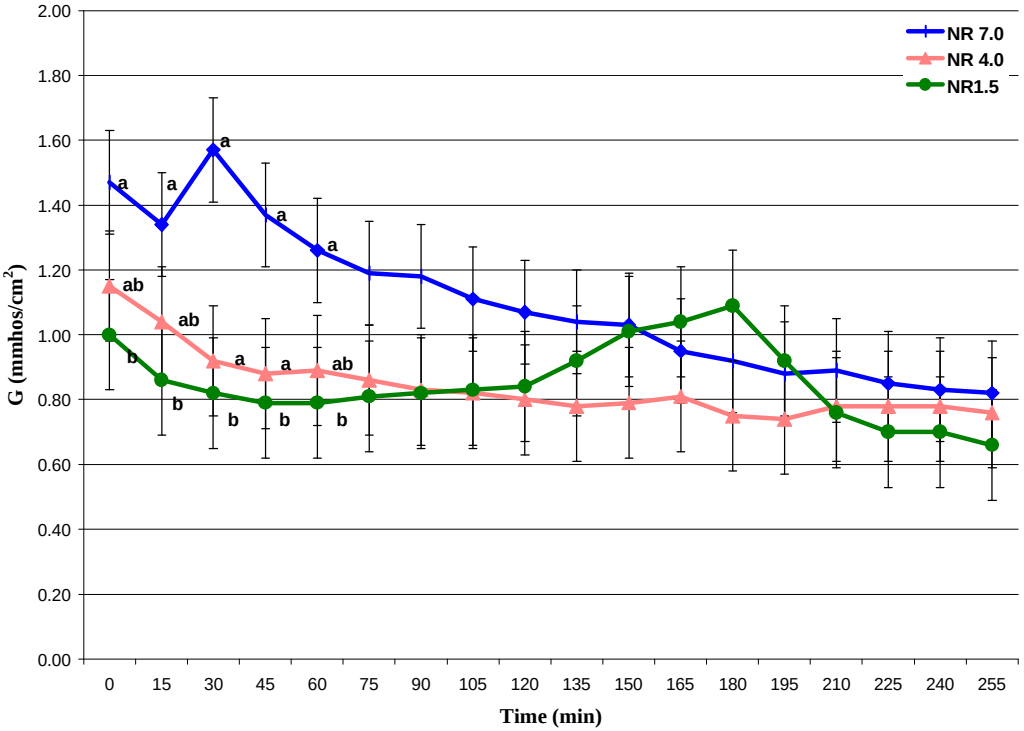


Figure 3-39. Mean Conductance: Normal Ringer's Solution

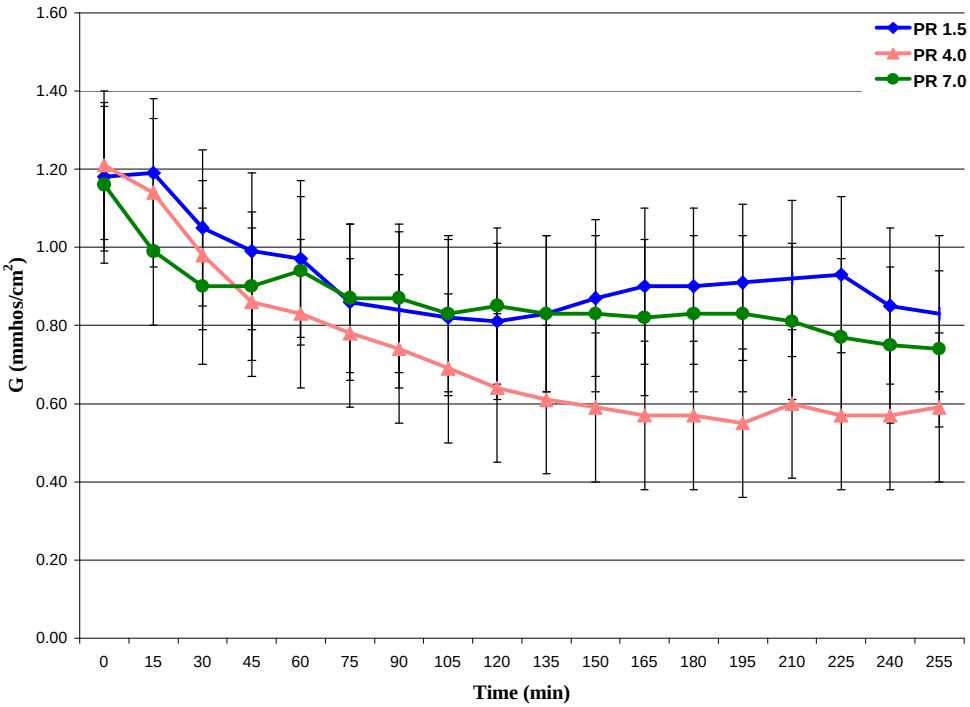


Figure 3-40. Mean Conductance: Propionic Ringer's Solution

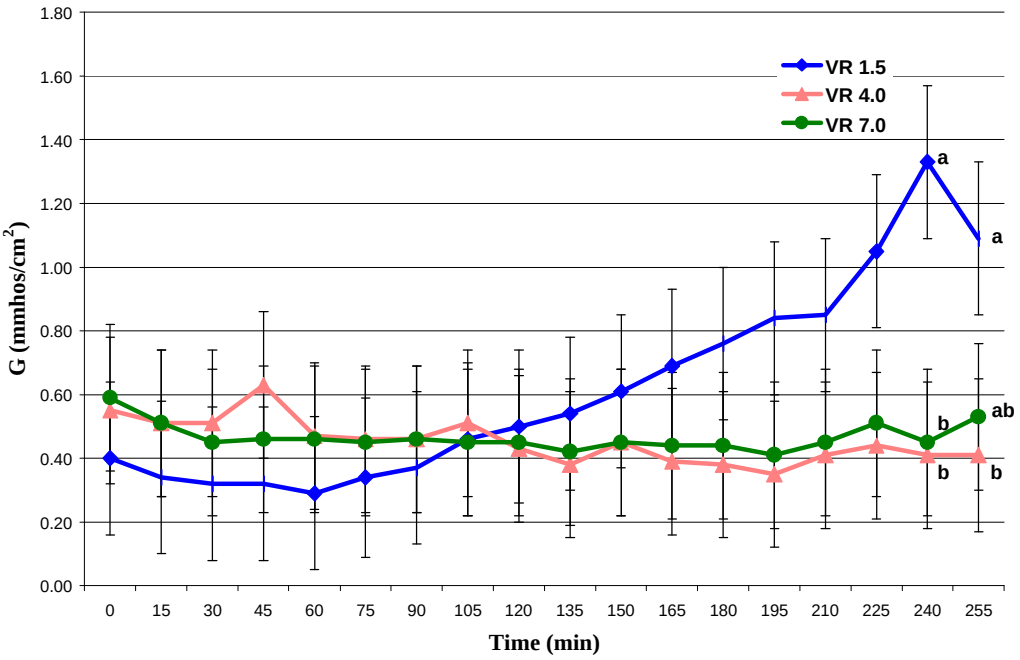


Figure 3-41. Mean Conductance: Valeric Ringer's Solution

Table 3-7. Effect of propionic Ringer's solution exposure on absorption into non-glandular mucosal tissue.

VFA	Mean absorption (mmols/L) ±SEM
Acetic (n=80)	0.16 ± 0.06[a]
Butyric (n=80)	0.27 ± 0.06[a]
Normal (n=82)	0.16 ± 0.06[a]
Propionic (n=84)	0.58 ± 0.06[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 3-8. Concentration of VFAs present after exposure to butyric Ringer's solution into non-glandular mucosal tissue.

VFA	Mean absorption (mmols/L) ±SEM
Acetic (n=80)	0.04 ± 0.06[b]
Butyric (n=80)	0.79 ± 0.06[a]
Normal (n=82)	0.05 ± 0.06[b]
Propionic (n=84)	0.06 ± 0.06[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 3-9. Effect of pH on butyric Ringer's solution exposure on absorption into non-glandular mucosal tissue.

pH	Mean propionic acid concentration (mmols/L) ±SEM
1.5 (n=112)	0.26 ± 0.05[a]
4.0 (n=106)	0.32 ± 0.05[a]
7.0 (n=108)	0.12 ± 0.05[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

by tissues exposed to butyric acid at pH 7.0 (Table 3-10).

Histopathologic examination of the non-glandular mucosa exposed to normal Ringer's solution at pH 7.0 showed no tissue changes and is shown for reference (Plate A-13). Tissues exposed to pH 1.5 had the highest percent of histologic change compared to tissues exposed to pH 4.0 or pH 7.0 (Table 3-11). Tissues exposed to valeric acid (Table 3-12, Plates A-23 and A-24) had the highest percent of histologic change compared to tissues exposed to propionic acid (Table 3-12, Plates A-14 and A-15) or butyric acid (Table 3-12, Plates A-16 and A-17). Normal Ringer's exposed tissues (Plates A-20 and A21) did not differ in percent of change compared to propionic acid and butyric acid or acetic acid (Table 3-12, Plates A-18 and A-19). The layers most severely affected ($P<0.01$) by histologic change were stratum basale and stratum spinosum combined followed by stratum spinosum, stratum basale, and stratum corneum, respectively (Table 3-13).

Table 3-10. Effect of treatment and pH after exposure on butyric acid absorption into non-glandular mucosal tissue.

VFA and pH	Mean absorption (mmols/L) ±SEM
AR 1.5 (n=27)	0.02 ± 0.10[a]
AR 4.0 (n=26)	0.05 ± 0.10[a]
AR 7.0 (n=27)	0.04 ± 0.10[a]
BR 1.5 (n=28)	0.96 ± 0.10[b]
BR 4.0 (n=27)	1.05 ± 0.10[b]
BR 7.0 (n=25)	0.35 ± 0.10[c]
NR 1.5 (n=29)	0.02 ± 0.09[a]
NR 4.0 (n=25)	0.09 ± 0.10[ac]
NR 7.0 (n=28)	0.03 ± 0.10[a]
PR 1.5 (n=28)	0.03 ± 0.10[a]
PR 4.0 (n=28)	0.10 ± 0.10[ac]
PR 7.0 (n=28)	0.04 ± 0.10[a]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 3-11. Effect of pH on non-glandular mucosal tissue change.

pH (n=241)	Mean percent of histologic change (%) ±SEM
1.5	32.94 ± 2.69[a]
4.0	28.06 ± 2.74[b]
7.0	25.79 ± 3.38[b]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.05$)

Table 3-12. Effect of treatment on non-glandular mucosal tissue change.

VFA Solution (n=241)	**Mean percent of histologic change (%) ±SEM**
Acetic	18.00 ± 4.43[a]
Butyric	28.51 ± 3.40[b]
Normal	25.79 ± 3.10[ab]
Propionic	30.52 ± 4.02[b]
Valeric	41.83 ± 3.49[c]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

Table 3-13. Differences in depth of penetration of non-glandular mucosal tissue change.

Zone (n=241)	**Mean percent of histologic change (%) ±SEM**
Stratum basale	19.13 ± 4.38[ab]
Stratum corneum	14.25 ± 4.31[b]
Stratum spinosum	26.58 ± 2.28[a]
Stratum basale and spinosum	55.76 ± 4.08[c]

SEM=standard error of the mean
Means not sharing like letters differ ($P \leq 0.01$)

CHAPTER 5

DISCUSSION

Butyric and propionic acids at an acid pH caused irreversible damage to the cellular sodium transport as reported in part I of this study. This part of the study will concentrate on changes seen with valeric acid, a VFA not previously reported. Results of this study suggest that valeric acid may induce functional and histologic damage at all pHs in the non-glandular mucosa of the horse stomach. In general, functional damage was manifested by a dramatic immediate decrease in sodium transport. This functional parameter was also associated with cell swelling in all layers of the non-glandular mucosa in many cases.

Explanation of changes in electrical measurements of the equine non-glandular mucosa is based mainly on studies in other organs and species. The values for short-circuit current and resistance in this study are similar to those found in rabbit esophageal epithelium, porcine non-glandular mucosa, and porcine colon.[1,5,8] This alludes to a similarity of diffusion barriers as well as ion transport mechanisms. Short-circuit current directly measures active ion transport and therefore is a parameter for epithelial function.[1] Previously it has been determined that short-circuit current is exactly equal to the net sodium transport.[9] Previous studies in frog skin,[10] rumen epithelium,[11] and rabbit esophagus[12] found that active sodium absorption accounts for short-circuit current. In rumen epithelium and rabbit esophageal epithelium, it was found that the living layers just below the stratum corneum function in sodium transport, powered by sodium pumps (Na^+K^+ATPases), in cells.[11,12] Recent studies in pig stomach have confirmed that current

flow across porcine non-glandular mucosa is due to sodium transport.[13]

In our study, valeric acid decreased ($P<0.01$) sodium transport at pH 1.5. Valeric acid decreased sodium transport the greatest of all VFA solutions, and had an immediate effect on short circuit current. Valeric acid exposed tissues had the lowest PCIsc values compared to acetic, butyric or propionic acid exposed tissues. Valeric acid decreased PD at this pH. Valeric acid exposed tissues decreased significantly in R and significantly increased in conductance. The decrease in resistance seen in tissues exposed to VRS may have been due to the severity of damage to the tissues (42%). The increase in conductance seen in valeric acid exposed tissues only supports this hypothesis since conductance increases as resistance decreases.

Electrical resistance is a barrier function of the horse stomach with electrical resistance ranging from 2-3000 ohms/cm^2 greater than forestomach epithelium.[12] 30 mmoles/L HCl concentration at pH 1.5 must be present to change R and result in histologic damage to pig and rabbit non-glandular mucosa when applied as long as 3 h to the mucosa *in vitro*.[1,14]

Our results differed from that of Orlando's group in which tissue treated with HCl at pH $\leq$ 1.5 had a slow decrease in tissue R preceding Isc change.[15-17] Isc was affected only when R decreased to $\leq$ 50% of its initial value. In our study, R began to decrease in valeric acid exposed tissues following the 30 min acclimation period. These results differ from those of Argenzio's group in which tissues exposed to acetate at pH $\leq$ 4.5 underwent a rapid and irreversible abolishment of Isc before any changes in R were detectable.[1] This implies that the non-glandular mucosa is highly permeable to the lipid soluble form of the weak electrolyte (acetate) and shows that acetate can penetrate the

tissue beneath even without changes in the permeability of the outer barrier. A sequence of tissue injury when VFAs are present was proposed, which follows. Initially, the undissociated acid penetrates the outer barrier layers and is taken up by living cells beneath the outer layers (Perhaps the VFAs are transported into the cells at the same time as sodium). Intracellular pH is higher in these cells compared to the extracellular fluid, so continued intracellular accumulation and dissociation of the weak acid acidifies the intracellular contents. This disrupts sodium transport (Isc) and cell volume regulation. Therefore, the continuous uptake of water and sodium across the basal membrane cannot be alleviated by basolateral sodium pumping or potassium leakage, resulting in cell swelling and necrosis. These occurrences undermine the outer barrier layers which leads to a secondary decrease in R. This implies that an H^+ induced decrease in R of the barrier function would enable sodium and water to enter into these underlying layers, hastening the process.

Tissues exposed to valeric acid show changes in short circuit current immediately after exposure. The tissues in this study appear to have been exposed to significant enough damage that they had advanced to the stage of a secondary decrease in resistance and an increase in conductance. Tissues exposed to pH 1.5 showed the greatest percent of change (33%) compared to pH 4 or pH 7 exposed tissues. Valeric (42%), propionic (31%) and butyric (29%) exposed tissues also showed the greatest percent of change that correlates well with Isc changes seen functionally.

At pH 4.0, valeric acid exposed tissues decreased sodium transport and consequently, decreased PCIsc. Valeric acid decreased PD. Unlike at pH 1.5, damage by valeric acid was not severe enough to cause the decrease in resistance and increase in

conductance seen at pH 1.5. Although tissues exposed to pH 4.0 were not as changed compared to tissues exposed to pH 1.5, (28% vs. 33%) there was still a significant amount of histologic change occurring with this pH. Tissues exposed to valeric acid seem to suffer functional and histologic damage at pH 4.0 as well as pH 1.5.

At pH 7.0, valeric acid exposed tissues decreased Isc. Valeric acid exposed tissues also decreased PCIsc and PD. Resistance was significantly higher in valeric acid exposed tissues. Conductance was significantly lower in valeric acid exposed tissues. This implies that, like valeric acid exposed tissues at pH 4.0, damage by valeric acid was not severe enough to cause the decrease in resistance and increase in conductance seen at pH 1.5. However, unlike studies of other VFAs, valeric acid exposed tissues at pH 7.0 showed functional and histologic damage. This could explain why some horses get ulcers even when acid suppressive agents are used to treat horses.

The histologic change that occurred was most severe at pH 1.5 compared to pH 4.0 or pH 7.0. The stratum basale and stratum spinosum layers together were most affected by damage (55% of damage) compared to either alone or the stratum corneum alone, showing that VFAs were able to penetrate and cause damage to the deep layers of the tissue.

ATPases have been histochemically located in equine stratified non-glandular mucosa.[18] In the stratum basale, high ATPase activity decreased as superficial layers were neared, showing that there is a gradient of metabolic activity in this mucosa similar to esophageal and rumen epithelium.

VFAs are absorbed at a rate which increases with chain length but not when the pH is neutral or basic.[19,20] This may explain why valeric acid is the most damaging VFA

compared to butyric, propionic, or acetic acid exposed tissues, respectively, at pH 1.5 and 4.0. However, the results from our study suggest that valeric acid may damage tissue even at pH 7.0. Valeric acid caused severe damage in tissue that decreased sodium transport, decreased resistance, and increased conductance. This acid damage leads to cell swelling and necrosis and may ultimately result in gastric ulceration.

Although a 60 mM concentration of acetic, butyric, propionic, or valeric acid was not found in the gastric contents of the horses studied, it is possible that the VFAs either volatilized just prior to collection or had already been converted to carbon dioxide, B-hydroxybutyrate, or acetoacetate. Some absorption did occur in the tissues exposed to propionic and butyric acids because tissues exposed to propionic and butyric acids had higher concentrations of these VFAs present at the end of the study compared to other VFAs. A study examined the mucosal uptake and serosal release of VFAs and used the difference between the parameters as an indicator for intraepithelial metabolism or VFA accumulation.[21] In this study it was found that when propionate (15 mmoles/L) was the only VFA present in mucosal solutions, mean mucosal concentrations decreased to about 13.5 mmoles/L and serosal concentrations increased to 0.8 mmoles/L, resulting in a mean mucosal loss of 18 μmoles/h and a mean serosal gain of 9 μmoles/h. Serosal release was equal to about 50% of mucosal uptake, and the authors felt that this indicated substantial intraepithelial metabolism or accumulation occurred during the experiment.

The results of this study suggest that valeric acid caused functional damage to the non-glandular mucosa at all pHs. This damage resulted in cell swelling and could eventually lead to gastric ulceration. The damage that occurs at pH 7.0 is concerning,

since no other VFAs have been found to cause damage at such a high pH. Valeric acid may be responsible for ulcer formation even when there is a high pH in the stomach.

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APPENDIX

Table A-1. Breakdown of horses in part I of the study by age.

Age	Number of horses
1	6
2	7
3	2
6	1
8	1
9	1
11	1
12	2
15	1
18	2
20	3
23	1
28	1
30	1
Total	**30**

Table A-2. Breakdown of horses in part I of the study by breed.

Breed	Number of horses
American Saddle Horse	3
Appaloosa	2
Arabian	3
Belgian	1
Mixed	1
Morgan x Arab cross	1
Paint	2
Paso Fino	2
Pony	1
Quarter Horse	4
Thoroughbred	1
Thoroughbred/Trakehner	1
Tennessee Walking Horse	8
Total	**30**

Table A-3. Breakdown of horses in part I of the study by sex.

Sex	Number of horses
Gelding	12
Mare	13
Stallion	5
Total	**30**

Table A-4. Breakdown of horses in part I of the study by reason for euthanasia.

Signalment	Number of horses
Behavior Problems	1
Cancer (carcinoma/melanoma/sarcoma)	2
Chronic Diarrhea	2
Chronic Pemphigous Foliaceous	1
Equine Protozoal Myoencephalitis	2
Joint Sepsis	1
Lameness	5
Laminitis	4
Neurological Damage	1
Old Age	1
Pituitary Adenoma	1
Recurrent Uveitis	3
Wobbler Syndrome	6
Total	**30**

Table A-5. Breakdown of horses in part I of the study by *Gastrophilus intestinalis* larvae present in the stomach.

With larvae	13
Without larvae	17
Total	**30**

Table A-6. Breakdown of horses in part I of the study by ulceration.

Horses With Ulcers	16
-Grade 1 ulceration	5
-Grade 2 ulceration	4
-Grade 3 ulceration	4
-grade not specified	3
Horses Without Ulcers	14
Total	**30**

Table A-7. Breakdown of horses in part II of the study by age.

Age	Number of horses
2	2
3	1
4	2
8	1
10	1
15	1
19	1
20	2
25	2
Total	**13**

Table A-8. Breakdown of horses in part II of the study by breed.

Breed	Number of horses
Mixed	5
Paint	1
Quarter Horse	2
Tennessee Walking Horse	4
None Specified	1
Total	**30**

Table A-9. Breakdown of horses in part II of the study by sex.

Sex	Number of horses
Gelding	5
Mare	6
Stallion	2
Total	**13**

Table A-10. Breakdown of horses in part II of the study by reason for euthanasia.

Signalment	Number of horses
Anorexia	1
Broken Femur	1
Diagnostic Evaluation	3
Equine Protozoal Myoencephalitis	3
Laminitis	2
Old Age	1
Weight Loss	1
Wobbler Syndrome	1
Total	**13**

Table A-11. Breakdown of horses in part II of the study by *Gastrophilus intestinalis* larvae present in the stomach.

With larvae	5
Without larvae	8
Total	**13**

Table A-12. Breakdown of horses in part II of the study by ulceration.

Horses With Ulcers	6
-Grade 1 ulceration	3
-Grade 2 ulceration	0
-Grade 3 ulceration	2
-grade not specified	1
Horses Without Ulcers	7
Total	**13**

Plate A-13. Normal tissue (100x).

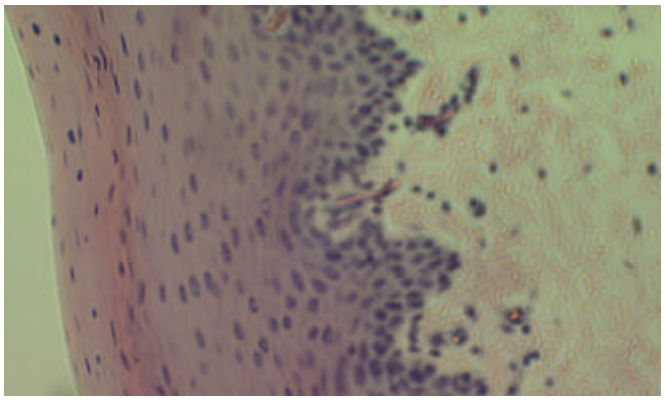


Plate A-14. Histologic change (100x) of non-glandular mucosa by PRS at pH 1.5.

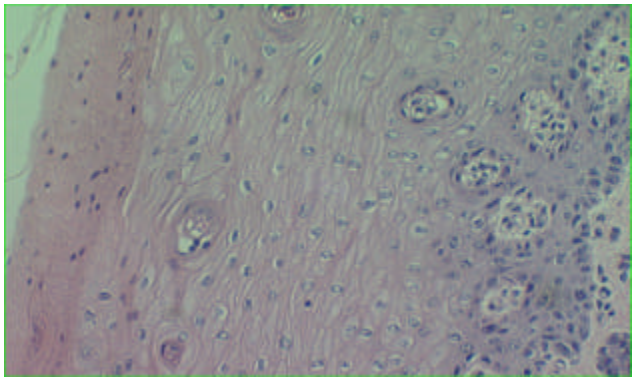


Plate A-15. Histologic change (100x) of non-glandular mucosa by PRS at pH 4.0.

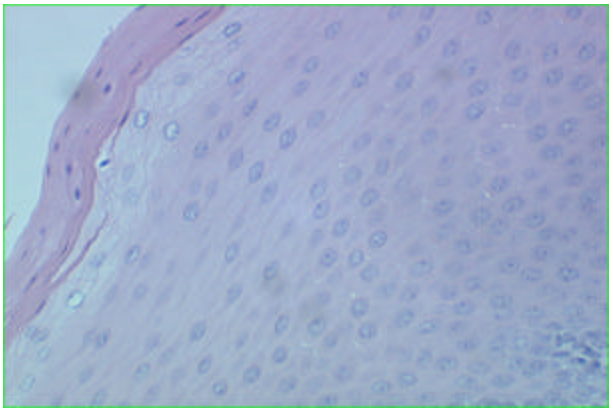


Plate A-16. Histologic change (100x) of non-glandular mucosa by BRS at pH 1.5.

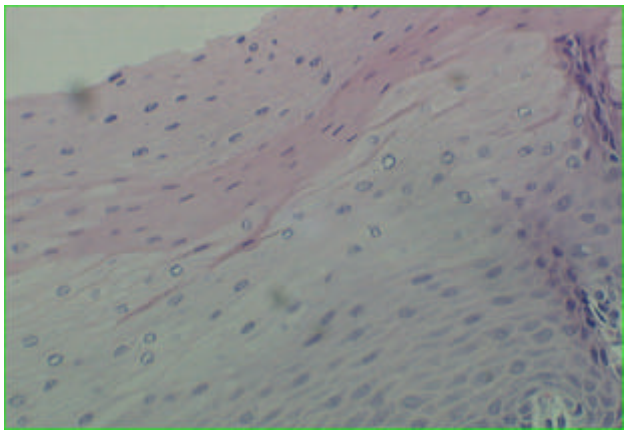


Plate A-17. Histologic change (100x) of non-glandular mucosa by BRS at pH 4.0.

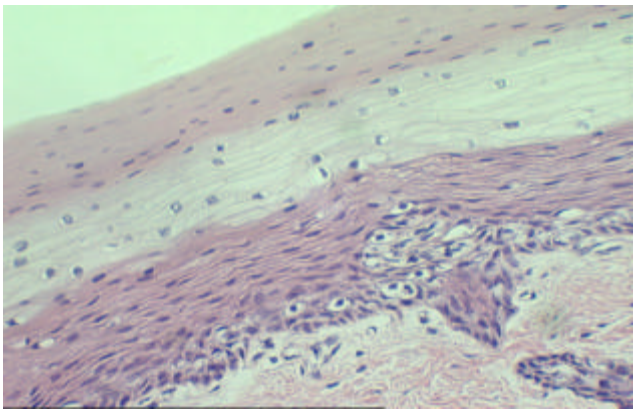


Plate A-18. Histologic change (100x) of non-glandular mucosa by ARS at pH 1.5.

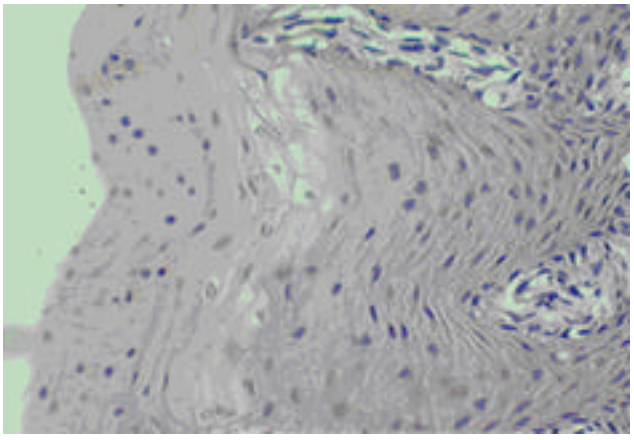


Plate A-19. Histologic change (100x) of non-glandular mucosa by ARS at pH 4.0.

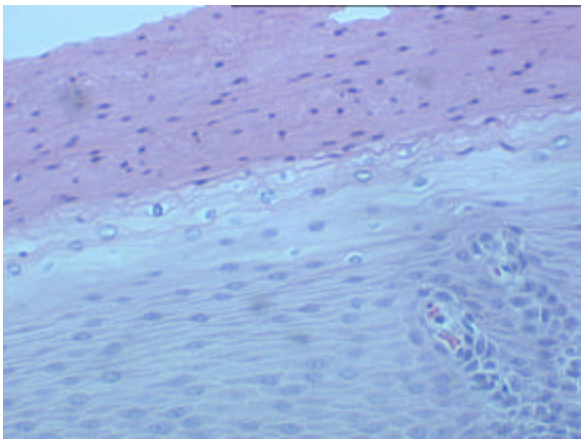


Plate A-20. Histologic change (100x) of non-glandular mucosa by NRS at pH 1.5.

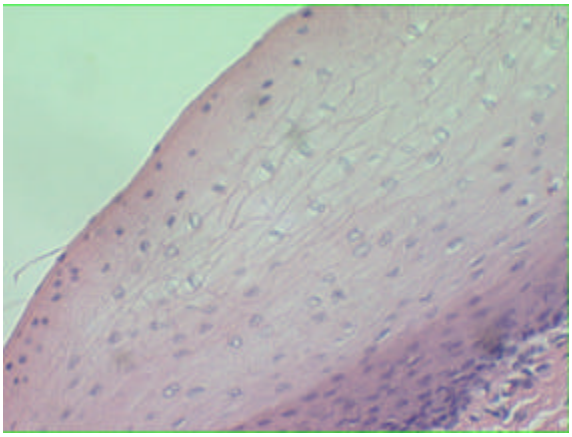


Plate A-21. Histologic change (100x) of non-glandular mucosa by NRS at pH 4.0

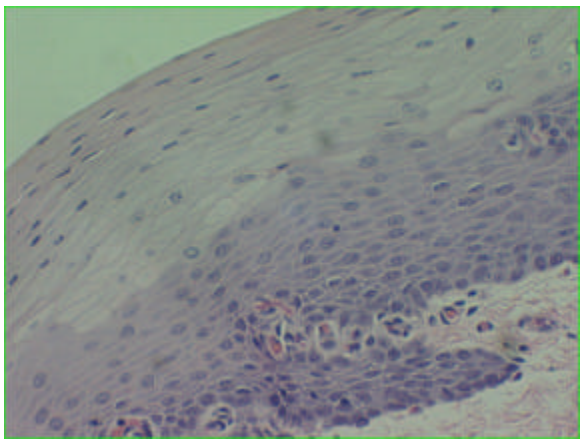


Plate A-22. Histologic change (100x) of non-glandular mucosa by VRS at pH 1.5.

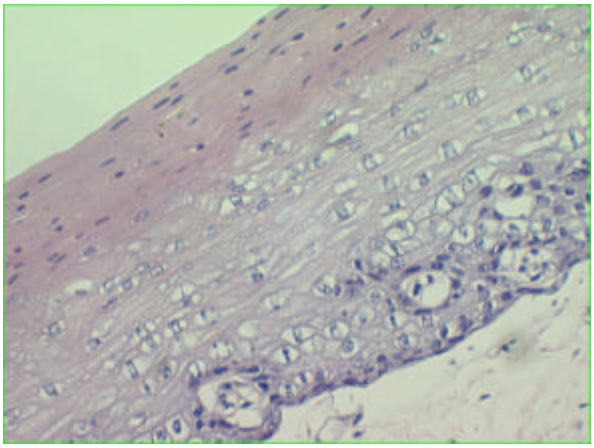


Plate A-23. Histologic change (100x) of non-glandular mucosa by VRS at pH 4.0.

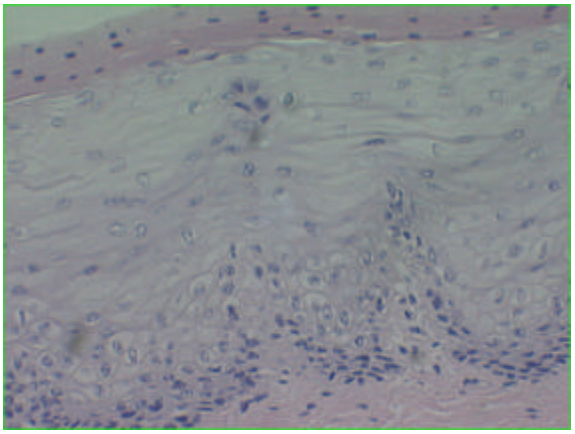
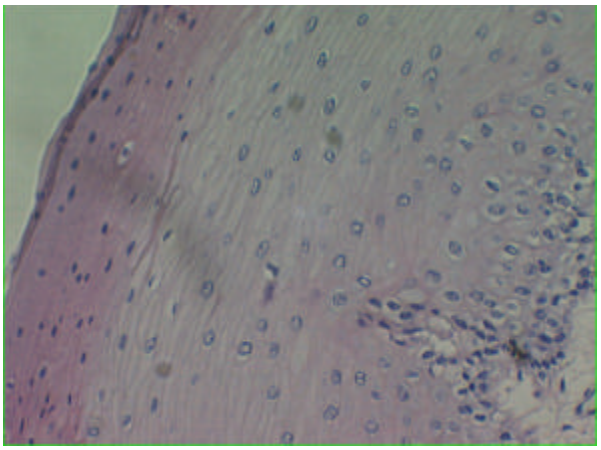


Plate A-24. Histologic change (100x) of non-glandular mucosa by VRS at pH 7.0.



VITA

Jenifer Ann Nadeau was born in Schenectady, New York on July 30, 1973. She graduated from Mohonasen Senior High School in June 1991. In August 1991, she entered the State University of New York at Morrisville College, where in May 1993, she received the Associate of Applied Science in Agricultural Science with an emphasis on Animal Science. She entered The University of Kentucky in August 1993, and received a Bachelor of Science degree in Animal Science in May 1995. In August 1995, she entered The University of Tennessee and received a Master of Science degree in Comparative and Experimental Medicine in August 1997. In August 1997, she began work on her doctoral degree in Animal Science at The University of Tennessee. She completed her degree in August, 2001. Jenifer has been hired by the Department of Animal Science at the University of Connecticut, in Storrs, CT. She will begin her career as a State Extension Horse Specialist at the end of August 2001.