THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF ANIMAL SCIENCE

Exploring Non-Invasive Analysis of Equine Gastrointestinal Health

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SPRING 2021

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree in Veterinary Biomedical Science
with honors in Animal Science

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ABSTRACT

The domestic horse relies on a diet high in concentrates to meet their energy needs as an athlete. This diet does not come without consequences to the health of the gastrointestinal tract. Ulceration and increased gut permeability (leaky gut), are common issues for the horse industry because of a diet high in non-structural carbohydrates. In this thesis, I will examine variability in the grading of equine ulcers and improved calculations of gastrointestinal permeability. Equine Gastric Ulcer Syndrome is apparent in both the squamous mucosa and the glandular region of the stomach. To determine the severity of ulceration in each anatomical region, a scoring system has been defined by the Equine Gastric Ulcer Council. This scoring system allows for consistency among researchers and veterinarians when analyzing the severity of ulceration in the different regions of the gastrointestinal tract through gastroscopy. Video endoscopies were evaluated by 5 different graders who were blinded to dietary treatments and replicated gastroscopies. A common scoring system was established, and 4 of the 5 graders attended a training workshop for training. Intra- and inter-grader reliability for the stratified squamous epithelium was then analyzed for each of the four stomach regions: greater curvature, lesser curvature, esophageal orifice, and body. Reliability within and between each grader was compared and although the scoring had relatively good reliability, there were differences in the median grades between the graders. The permeability of the gastrointestinal tract is critical to maintain a healthy animal. The need for a test to detect changes in the permeability of the gastrointestinal could have widespread application for further understanding gastrointestinal disorders, especially in the equine industry. A four-sugar absorption test was used to measure gastrointestinal tract

permeability. After administration of an oral dose of the sugar marker, the four sugars analyzed were sucralose, sucrose, mannitol, and lactulose. This paper reviews the importance of developing consistent definitions of equine gastrointestinal disease and the value of the 4-sugar absorption test for estimating gastrointestinal health in equine athletes.

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LIST OF ABBREVIATIONS

BP	Balancer Pellet
BW	Body Weight
CBC	Concentrated Bovine Colostrum
CF	Correction Factor
DM	Dry Matter
ECL	Enterochromaffin-like
EGGD	Equine Glandular Gastric Disease
EGUS	Equine Gastric Ulcer Syndrome
EO	Esophageal Orifice
ESGD	Equine Squamous Gastric Disease
GC	Greater Curvature
GI	Gastrointestinal
GC-FID	Gas Chromatography with Flame Ionization Test
GIT	Gastrointestinal Tract
HA	Hay Only Diet
HCL	Hydrochloric Acid
HCLF	High Concentrate Low Forage Diet
HO	Hay and Oat Diet
HOP	Hay, Oat and Pellet Diet
HP	Hay and Pellet Diet
IBD	Inflammatory Bowel Disease
ICC	Intraclass Correlation Coefficient
LC	Lesser Curvature
LCHF	Low Concentrate High Forage Diet
NC	No Colostrum
NSC	Non-Structural Carbohydrate

SAT	Sugar Absorption Test
VFA	Volatile Fatty Acid
WBC	Whole Bovine Colostrum

ACKNOWLEDGEMENTS

I would like to thank everyone at Penn State and in the College of Agricultural Sciences who has helped me over the past four years. I would especially like to thank Dr. Staniar who has been a mentor and teacher with not only equine nutrition and research, but with life. Working with him over last few years, he has pushed me to reach new goals and visions I did not even know I could. Without his dedication and passion for research and teaching I could not have completed these projects and furthered my knowledge. I would also like to thank Kathryn Norris, Siga Lapinskas, and Hannah Van Every, who without, this research could not have been further studied. I would also like to thank my family and friends who have motivated me and consistently supported me through all of my goals.

Chapter 1

Gastrointestinal Health in Horses – A Review

1.1 Introduction

Domesticated over 6,000 years ago, horses were often maintained in environments where their diet and feeding behavior were changed due to the fact that they were living with humans (Goodwin, 2007). Following thousands of years of selection for recreation, work, and sporting, the horse now has a higher energy requirement than that of its ancestor to meet their performance requirements. Domestication of horses led to a shift in feeding strategy from 14-16 h of grazing a myriad of different forages, to 8-10 h of grazing and meal feeding one forage and a concentrate. This likely results in a mismatch between feeding strategy and gastrointestinal physiology (Cooper, 2005).

Three main adaptations of horses over nearly 60 million years of evolution relate to the changing environment in which those horses survived; body size, limb characteristics, and teeth (Janis, 1976). Body size increased which allowed for an increase in gut contents, larger gastrointestinal (**GI**) tract, and allowed for more space and time to digest the feed. Distal limbs increased in size which allowed for better adaptation to a grazing lifestyle and allowed greater ease in moving from place to place (Janis, 1976). The adaptation of hypsodont teeth, which continuously erupt throughout their lives, allows for continual grinding of forage (Janis, 1976).

Their gastrointestinal tract evolved to be capable of digesting starch, protein, and fat efficiently in the small intestine, with main fermentative capacities in the cecum and large colon of the hindgut (Ralston, 2007). Management practices of most horses kept today include being

fed fewer meals with a greater amount of starch and little forage (Davidson and Harris, 2007). The purpose of this review is to highlight how the anatomy and physiology of the gastrointestinal tract and the diet of the horse are intertwined, and how these play a role with common health issues.

1.2 Nutritional history of equine species

Before domestication, the horse freely roamed the wide grasslands of North America and possibly Central Asia and eastern Europe (Janis, 1976). As their adaptation from browser to grazer progressed, the horse became better suited for a high-fiber, low quality forage diet, as opposed to their original low-fiber, high-quality forage diet (Edouard, 2008). Domesticated horses performing various athletic activities require higher quality forages. These broadly include less mature grasses or even legumes that have a lower fiber and lignin content, i.e. more digestible, and greater nutrient and energy content. The economic and geographic constraints of the increase in domesticated horses of the US, led to the confinement of these animals in stalls. Stable management techniques used the efficiency of concentrated feedings of grain and hay as opposed to a continual grazing (Clarke, 1990). These highly concentrated, low forage diets, although providing the energy the athletic horse needed, increased the risk of gastrointestinal health complications (Davidson and Harris, 2007).

Both forages and concentrates play an important role in the equine diet. Because of the horse's evolutionary history, forages remain an important part in the diet. Most hays high in digestibility allow for increased chewing and saliva stimulation and a greater absorption of nutrients (Janis 1976; Davidson and Harris, 2007). These plant-based diets are then fermented in

the hindgut by microbes that reside in the gastrointestinal tract. These microbes digest fiber and create volatile fatty acids (**VFA**) as a byproduct of fermentation. Hindgut fermentation can provide up to 70% of their energy requirements (Harris, 2017). The energy requirements of the work and performance horse are often not able to be met by forages alone and instead concentrates are added to the diet. Concentrates included cereal grains such as oats, corn, and barley. These grains are high in non-structural carbohydrates (**NSC**), and at high levels in the diet can cause various gastrointestinal health disruptions that will be discussed later (Nadeau, 2000). High concentrate diets, high in digestive starch reduce gut health in the horse.

While there are potential problems created by a high starch diet throughout the GI tract, it makes sense to start at the beginning, the stomach. A prevalent gastrointestinal health issue in performance horses is gastric ulceration (Andrews 2017). According to the Equine Gastric Ulcer Council, a gastric ulcer is defined as an alteration in of the gastrointestinal mucosa that destroys cellular elements (Andrews, 1999). Because of the multifactorial nature of this disease, it has been named equine gastric ulcer syndrome (**EGUS**). One effective method of reducing the risk or prevalence of EGUS is increasing the amount of forage consequently stimulating chewing and increasing saliva production which has a buffering capacity on the acids produced in the stomach (Videla, 2009). Detection of ulceration is mainly observed with the use of a gastroscopy; however, this is impractical as a wide-spread screening test because many horses affected by EGUS do not show clinical signs (Heweston, 2006). The need for a universal, relatively inexpensive, and user-friendly test to determine presence of ulceration in the horse's gastrointestinal tract is needed.

1.3. Equine Gastrointestinal Tract Anatomy and Physiology

The digestive system of the horse is well-adapted to their continuous consumption of a forage-based diet. From the mouth to the ascending colon, each part of the gastrointestinal tract (GIT), will be described below, as well as the necessary mechanisms and functions that occur in each. The relationship between the anatomy and physiology of the GIT and the diet is critical for maintaining the health and wellbeing of the animal.

1.3.1. Mouth

The horse's skull anatomy represents adaptation to grazing. Examples include huge flat bones for muscle attachment on the maxilla and mandible; hypsodont type teeth that continue to erupt for much of the horse's life, lips and incisors that facilitate great precision in the choice of forages consumed (Davidson and Harris, 2007). Once food is in the mouth, the horse uses an orbital jaw motion and a large powerful tongue to form a bolus that is moved towards the back of the mouth. Using the screw-like palatine ridges along the roof of the mouth, while at the same time chewing stimulates copious amounts of saliva production to aid in lubrication of the bolus as well as some digestive function (Baker, 2002). It is well-established that the more a horse chews, the more saliva that it will produce (Davidson, 2007; Meyer et al., 1995). Finally, the pre-molars and molars significantly reduce particle size of food prior to it being swallowed.

1.3.2 Stomach

The feed exits the mouth through the esophagus, a long muscular tube, to the stomach where initial digestion occurs. The stomach is divided into 2 distinct anatomical regions, the non-glandular (dorsal) and glandular (ventral) regions, and the margo plicatus separates the two (Andrews, 1999). The non-glandular region makes up about one third of the equine stomach, lacks glands, and is covered by stratified squamous epithelium. Most ulceration occurs in this region (Andrews, 1999.). In the stratified squamous mucosa there has been no evidence found for active transport of substances such as bicarbonate or HCl, which would lead to acid secretion (Merritt, 1999). The anatomical regions can be seen in Figure 2.

The other two thirds are made up of the glandular portion of the stomach and secretes hydrochloric acid, pepsin, bicarbonate, and mucus (Andrews 1999). The glandular portion is divided into three regions; the cardiac, fundic, and pyloric. Little is known about the functionality of the cardiac region of the horse (Bell, 2007). The fundic and pyloric regions are responsible for secretion of HCl, pepsinogen, gastric lipase, and a thick mucus protective layer (Andrews, 2017; Merritt, 1999). The continuous acid secretion that occurs in the glandular region of the horse is stimulated by gastrin, histamine and acetylcholine. Other enzymes such as pepsin, also play a role in the acidic environment and contribute to the low pH in this region, as opposed to the more basic pH in the non-glandular region of the stomach (Reese, 2009).

Two types of secretory cells are found in the glandular region of the stomach; parietal and chief cells. Parietal cells in the glandular portion secrete HCl and zymogen (chief) cells secrete pepsinogen into the gastric lumen. Hydrogen ions are secreted using an apical membrane bound H+, K+ pump that converts ATP to ADP. While H+ is secreted in the lumen, chloride ions are also secreted into the lumen by a K+, Cl- co transporter (Murray, 1992; Merritt 1999).

Parietal cell acid secretion is regulated by three main factors; gastrin, histamine, and acetylcholine (Reese, 2009). Gastrin is released in the fundus and antrum by gastric G cells which stimulate calcium-mediated pathways resulting in an increase in acid secretion. Enterochromaffin-like (**ECL**) cells secrete histamine, which stimulates the secretion of acid from parietal cells (Merritt, 1999). The final factor affecting parietal cell acid secretion is acetylcholine. Somatostatin is released by D cells in response to a decreased pH in the lumen of the stomach. This in turn inhibits the release of HCl by inhibiting gastric G cells (Murray, 1992). Chief cells secrete pepsinogen. Pepsinogen is converted to pepsin under environmental conditions with a pH of <3.0 and initiates the digestion of protein (Murray, 1992).

The pH of the stomach differs depending on the anatomical region. In the dorsal portion of the stomach, the non-glandular region, a near neutral pH can be found (pH 5-7). Closer to the margo plicatus, the pH becomes more acidic (pH 3-6). The pH becomes even lower in the glandular region where most of acid is secreted (pH 2-4) extending to the pylorus (Andrews and Nadeau, 1999; Merritt, 2003). As feed enters the stomach fermentation and hydrolysis begin with most absorption and digestion of proteins, fats, and carbohydrates occurring in the small intestine (Ericsson, 2016).

1.3.3. Small Intestine

The main function of the small intestine is digestion and absorption of nutrients. The small intestine is characterized by having a numerous villi and crypts which contribute to its large surface area (Thompson, 2019) The villi of the small intestine extend into the lumen and

this intersection can be referred to as the crypt-villus axis (Muglia, 2011). The small intestine is also home to absorptive enterocytes, goblet cells and Paneth cells that play a role in the absorptive and regulatory functions of the small intestine (Gonzalez, 2015). Tight junctions, which will be further explored in the Gastrointestinal Permeability section, are essential for maintaining proper function of the small intestine.

The small intestine is separated into three components; duodenum, jejunum, and ileum. The duodenum is the first section of the small intestine and is composed of glandular tissues and is the site of bile emptying (Moore, 2001). Digestion of fats, proteins and carbohydrates also occurs here (Thompson, 2019). The main function of the jejunum, which is rich in vasculature, is the absorption of nutrients. The ileum is the final portion of the small intestine and has many similar functions to that of the jejunum and can be recognized by the large amount of lymphatic tissue that resides here (Moore, 2001).

1.3.4. Hindgut

The particles that cannot be absorbed and digested in the small intestine then move into the hindgut composed of a highly sacculated cecum and a segmented colon, accounting for approximately 62% of the entire GIT volume (Reed, 2021). The cecum is a muscular blind sac and is mainly responsible for water absorption. The colon is composed of different segments separated by important flexures: right ventral colon, sternal flexure, left ventral colon, pelvic flexure, left dorsal colon, diaphragmatic flexure, and the right dorsal colon (Moore, 2001).

The ileum enters the cecum at the ileocecal orifice located near the ceco-colic junctions. The cecum traps large particles at the cecal base and contracts pushing some particles to the right

ventral colon. This retention of coarse particles allows for liquid and fine particles to be moved to the left and right dorsal colon (Santos, 2011). From the right dorsal colon, feed is then passed to the transverse and small colon. Here most of the feed has been digested already and the whatever feed is not able to be digested will be formed into fecal balls and passed to the rectum. Water reabsorption is also an important function of the colon. Up to 95% of the total fluid in the GIT is reabsorbed by the cecum and colon (Moore, 2001).

The hindgut is the main site of fermentation in the horse as well as home to most of the microbial population of the GIT (Santos, 2011). The microbial populations that reside here are responsible for fermentation of all structural carbohydrates which produce volatile fatty acids (**VFA**) as a byproduct. The horse seems capable of meeting between 30 and 70% of its maintenance energy requirements from VFAs (Rowe, 1994). High concentrate diets decrease intestinal pH disrupting the microbial populations that reside here. These diets are often high in starch, which when rapidly fermented in the hindgut, results in a decrease in pH. When fermented starch produces lactic acid and CO_2. Buildup of lactic acid and low pH can disrupt the microbial population and alter VFA absorption (Daly, 2011; Rowe, 1994). Understanding the anatomy and physiology of the GIT is important in understanding how the diet plays a role in the gastrointestinal health of the horse.

1.4. Gastrointestinal Disease of the Horse

1.4.1. Gastric Ulcers

1.4.1.1. Importance and Prevalence

Equine gastric ulcer syndrome (**EGUS**) is a term for describing gastric ulceration in horses. This term however, requires specification in regard to the region of the stomach that is being described. Equine Squamous Gastric Disease (**ESGD**) and Equine Glandular Gastric Disease, (**EGGD**), describe ulceration in the two regions of the equine stomach (Sykes, 2015). Both are serious issues in the equine industry; however, much remains unknown about glandular ulcers and the rest of this review will focus on ESGD (Andrews 2017).

Gastric ulceration can occur in horses of all breeds and ages making it important for horse owners, veterinarians, and the equine industry, to understand the causes and signs of EGUS. Although clinical signs vary, many result in poor health and decreased athletic performance. Decrease in performance is associated with a decline in income for those in the racing industry (Heweston, 2006).

The greatest prevalence of ESGD is in Thoroughbred racehorses with 37% of untrained horses affected, increasing to 80-100% within 2-3 months of training. A similar prevalence occurs in Standardbred racehorses as well. In one study, up to 17-58% of show/sport horses and 37-59% of pleasure horses were affected (Sykes, 2015). The increasing intensity of exercise and management is a factor contributing to ulceration; however, it is still prominent in leisure horses (Andrews, 1999). It is important to understand the risk factors and proper management and prevention techniques to reduce the prevalence of ulcers in the equine species.

1.4.1.2. Risk Factors

Horses are continual acid secretors with a low pH of about 3.0, in the ventral portion of the stomach (Sykes and Jokisalo, 2015). Excessive exposure of the stratified squamous epithelium to acid is thought to be a primary cause of ESGD. The pH of the stomach can be affected by the diet provided to the horse. In one study, stomach pH was around 4.0 in horses fed alfalfa hay and grain, compared to a pH of 2.0 in horses fed Brome grass hay, until 5 hours after eating. This may have been due to a high concentration of calcium in the alfalfa hay-grain diet (Nadeau, 2000). Hydrochloric acid (**HCl**) is not the only acid contributor in the stomach; VFA, lactic acids and bile acids, as well as enzymes, such as pepsin, contribute to the low pH environment. In some studies, the presence of excessive acids and enzymes, especially in a low pH environment, have led to ulceration; however, a few studies have also resulted in no significant effect, determining the specific role of these compounds needs further investigation (Andrews 2017). Although overproduction of acid and low saliva production are the primary causes, there are several other risk factors involved.

Horses that are worked at a trot, canter, or gallop for most of their daily workouts are at a heightened risk of developing EGSD (Sykes, 2015). A study showed that horses running on a high-speed treadmill have increased abdominal pressure and decrease stomach volume (Reese, 2009). This contraction of the stomach allowed the acid from the glandular mucosa to reflux into the non-glandular mucosa which would increase the ulceration, according to the author (Reese, 2009).

Horses that graze on pasture have a decreased prevalence of EGUS. One proposed theory is that during grazing there is a continuous flow of saliva that buffers the stomach acid (Videla, 2009). Horses kept in stables; however, have altered eating habits as well as induced stress,

increasing the rate of acid clearing in the stomach (Hepburn, 2011). Meal-fed horses often have longer periods of the day during which they are not consuming food. Horses continuously secrete acid and as the previous meal moves out of the stomach, the pH will drop and contain less food in the stomach. This leaves the squamous region exposed to a lower pH for longer (Videla, 2009).

Increased starch / grain intake may also lead to increased risk of ESGD (Sykes, 2015). Serum gastrin concentrations are high in horses fed a high grain diet. HCl secretion occurs with an increase in serum gastrin, resulting in acid damage to the glandular region (Reese, 2019). Low NSC diets help to produce a greater fiber mat composition in the stomach that provides a physical barrier, whereas diets with high NSCs are ingested more quickly in horses with ulcers and with less saliva production and are fermented rapidly by bacteria, and results in production of VFAs. VFAs in the presence of a low pH environment can cause damage to the non-glandular mucosa (Andrews 2017; Reese 2009).

1.4.1.3. Clinical Signs

Although the range of clinical signs of gastric ulceration are vague, the most common signs include: poor performance, changes in behavior, decreased appetite, poor body condition, weight loss and colic (Sykes 2015; and Andrews 1999). Because poor performance can be due to factors other than ulceration, such as diet and management, it is often difficult to directly test. A study done with Thoroughbred racehorses did find a direct link between gastric ulcers and decreased performance, however the mechanism by which this occurs has not been established (Franklin, 2008). In the 2008 Franklin study, 4 racehorses were tested in which their only

abnormality was EGUS. These 4 horses were treated with omeprazole, a gastric acid inhibitor, and showed improvement in performance with each case suggesting to the authors a link between gastric ulcers and performance (Franklin, 2008). Ulceration has also been associated with behavioral changes such as reduced appetite, nervousness, aggression and self-mutilation (Sykes, 2015). Reduced appetite may range from mild to severe and may even be unrecognized in horses with gastric ulcers. Poor body condition is commonly associated with gastric ulcers in horses in active training (Sykes, 2015; Andrews, 1999). Abdominal pain is also associated with gastric ulcers and the severity of one, often affects the other (Rabuffo, 2009). Although these are common signs of EGUS, not all horses who have ulceration show all, if any, of these signs, and gastric ulceration should be confirmed by gastroscopy (Sykes 2015).

1.4.1.4. Diagnosis

The numerous clinical signs and range of severity of ulceration, make it difficult to base diagnosis solely off these signs. Gastroscopy is the only definitive diagnosis for gastric ulcers (Videla 2009). Gastroscopy requires a 3-m endoscope and the horse to be fasted. The timing of fasting may depend on the athletic nature and the diet of the animal, for example a sport and pleasure horse on a standard hay-based diet, should have a minimum of 16 hours of fasting to ensure an empty stomach. However, racehorses on a high grain/ low roughage diet should have about 6-8 hours of fasting. Horses are usually sedated for the duration of the gastroscopy. Optimally, both the non-glandular and glandular regions should be examined and scored. Under perfect conditions it is even possible to examine the very proximal section of the duodenum (Sykes and Jokisalo, 2014).

Once the endoscopy is completed a scoring system can be used to grade the ulceration at the various anatomical regions. The 1999 Equine Gastric Ulcer Council created a grading scale for the non-glandular region which ranged from a grade of 0, epithelium intact and no appearance of hyperemia or hyperkeratosis, to a grade of 4, extensive lesions with areas of apparent deep ulceration (Andrews, 1999). The entire grading system can be seen in Figure 1. This scoring system has been used by many researchers and veterinarians studying ulceration, however many continue to create their own scoring systems making it difficult to compare ulceration among different studies.

The glandular region has a separate scoring system. Rather than a numerical based score, the glandular region is often graded based on the appearance of lesions. A lesion is an area of tissue damage and it is these areas that are then used for determining ulcer severity (Sykes, 2015). A 1997 study by MacAllister created a system based on the appearance of the glandular lesions. The lesion severity scores and descriptions are as follows; 0: no lesion; 1: appears superficial (no mucosa missing); 2: deeper structures involved than No. 1; 3: multiple lesions and variable severity (1, 2 and/or 4); 4: same as 2 and has active appearance (active = hyperaemic and/or darkened lesion crater); 5: same as 4 plus active hemorrhage or adherent blood clot (MacAllister, 1997). The use of this scale enables comparison among clinicians and allows for monitoring healing and treatment (Reese, 2009).

1.4.1.5 Treatment

One form of treatment for EGUS is the use of pharmaceuticals. The most common classes of drug used in the horse includes proton pump inhibitors and H_2 receptor antagonists.

Both suppress acid produced and allow for an increase in gastric pH (Sykes, 2015). Omeprazole is a proton pump inhibitor used in horses. It is an inhibitor of gastric acid secretion and works by blocking the H+, K+ATPase pump in the secretory membrane of parietal cells (Andrews, 1999). In one study, omeprazole given intravenously increased the basal gastric pH within 2 hours of administration in adult horses, however, pH returned to basal levels after 8 hours (Bell, 2007). In another study, gastric acid secretion decreased 27 hours after the last of five intravenous doses of omeprazole (Bell, 2007). Omeprazole administered once daily typically yields 24 hours of acid suppression (Sykes, 2015a). The most common histamine receptor antagonists studied in the horse include cimetidine and ranitidine (Andrews, 1999). Cimetidine and ranitidine block histamine from the H_2 receptors of the parietal cell decreasing HCl secretion (Bell, 2007).

There are other treatments that are less expensive than pharmaceuticals such as feed supplements and antacids. Dietary management is an important aspect of managing ulceration. To keep the pH of the stomach higher it is important to have readily accessible alfalfa hay, or allow pasture turnout (Buchanan, 2003). Antacids, while having previously been used, are impractical due to the frequency of administration (6-12 times a day) (Andrews 1999). It is thought that aluminum containing antacids may aid in mucosal protective effects, however their effect on controlling pH remains unclear (Buchanan, 2003). It is important to combine dietary management, exercise, and use of pharmaceuticals when reducing the severity of ulceration.

1.4.1.6. Summary

Equine Gastric Ulcer Syndrome is very common among the horse industry and should be taken seriously. It is important to recognize common clinical signs; however, it is also important

to understand that not all horses with ulceration will show signs and gastroscopy should be used for proper diagnosis. The scoring system commonly used among researchers and veterinarians is helpful for diagnosis, however uniformity may not be seen across all users. Certain pharmaceutical drugs, such as omeprazole have been shown to be effective, however proper management is needed to maintain long term GIT health in the horse.

1.5.2. Gastrointestinal Permeability

1.5.2.1. Gastrointestinal Barrier

In numerous mammalian species nutrients, electrolytes and water are actively or passively absorbed from the GIT lumen across the epithelium into the organism. The integrity of the gastrointestinal tract lining is critical in maintaining the health of an animal. There are a variety of cells connected by intercellular tight junctions, that make up the epithelium (Bjerknes, 1999). The epithelium is the largest mucosal surface in the body and is composed of a single layer of cells (Stewart, 2017). The enterocytes that line the GIT have a lipid bilayer plasma membrane that contains transport and tight junction proteins, all of which is important in controlling permeability. Transporters, usually proteins, are needed throughout and regulate the movement of water-soluble molecules (Arrieta, 2006). The two main routes across the epithelial are by transcellular and paracellular transport (Powell, 1981). Transcellular transport consists of formation of apical vesicles that pinch off and move through the cell to the basolateral cell membrane whereas paracellular transport across tight junctions and via the intracellular space

(Stewart, 2017; Powell, 1981). The paracellular pathways are also regulated by adherens junctions which provide strong connective bonds between epithelial cells (Stewart, 2017).

Tight junctions are on the apical-lateral membrane between enterocytes. The tight junctions create a barrier in which only small molecules are a certain size can flow through. The tight junctions of the epithelial become more permeable in response to stimuli such as diet, humoral or neural signals, inflammatory mediators, and mast cell products (Arrieta, 2006). Tight junctions are responsible for maintaining low permeability to solute transport. One transmembrane protein important in tight junction formation is claudin. Claudins have two main functions: barrier function in sealing the extracellular spaces between cells, and forming channels that control which ions can selectively cross the paracellular space (Wang, 2017). If these interactions are disrupted, potentially noxious molecules may enter into the lumen resulting in immune activation and inflammation, and may cause intestinal diseases (Stewart, 2017).

The interaction of mucus and a water layer forms a diffusion intestinal barrier. The mucus prevents bacterial adhesion and protects villi. Phospholipids, tight junctions, lymphocytes, and gut microbiota are all other components that contribute to the intestinal barrier (Stewart, 2017). Barrier function and assembly are both regulated by signaling pathways that include tyrosine kinases, Ca^{2+}, and protein kinase C (Anderson, 1995). Histamine and α-thrombin increase permeability by inducing gaps between the cells through disruption of tight junctions (Anderson, 1995). A healthy epithelium is impermeable to toxins, pathogens, and antigens, and remains selectively permeable for the absorption and transport of ions, nutrients, and water (Stewart, 2017). Abnormal / altered intestinal permeability is present in human patients with inflammatory bowel disease, (**IBD**) caused by excess IL-6 production (Delzenne, 2011). This is also known as “leaky gut” and is also seen in diabetes patients (Bruewer, 2003; Delzenne, 2011).

This leakiness can be described as a failure of the epithelium's barrier function and can be tested using permeability tests.

1.5.2.2. Sugar Absorption Tests

In both human and animal tests, intestinal permeability has been assessed through use of urine and serum recovery of radio labeled markers, PEG, and sucrose markers both in vivo and in vitro (Stewart, 2017). Sugar absorption test allow for a measure of intestinal permeability however have not been extensively researched. Sucrose is a common sugar used to test permeation. It is inexpensive, non-toxic and has a specific outcome in the small intestine due to its size. Once hydrolyzed, sucrose is concentrated in the urine. If found in increased amount in blood or urine, this would suggest gastrointestinal damage (Hewetson, 2006). Administering sucrose to test for gastric ulcers has been seen throughout the equine industry (Hewetson, 2017; Hewetson, 2006; O'Conner, 2004).

The selective nature of the gastrointestinal tract transport allows for the use of sugar absorption test to measure intestinal permeability, non - invasively. Common sugars that are utilized in sugar absorption tests (SAT), include sucrose, lactulose, mannitol, rhamnose, and sucralose (VanElberg, 1995; Arrieta, 2006). Interpreting the absorption and non-absorption of these sugars can be difficult. Transport rate for example, depends on the size of the molecule. The smaller molecules, mannitol and rhamnose, can pass through selectively permeable channels between tight junctions, whereas the larger disaccharides can be absorbed deeper in the crypt when the channel size has increased, resulting in damage epithelial (Arrieta, 2006).

1.5.2.3. Implications in the Horse

The use of a sugar absorption test in the horse has not been widely used to study gastric ulceration (Hewetson, 2006; O'Connor, 2004). The use of the sugars sucralose, lactulose, sucrose and mannitol are the most common. These sugars are used because they are easily measured in urine and serum, are not radioactive, and can provide information about the permeability in different sections of the gastrointestinal tract (Shaikh, 2015). Using a SAT in the horse would allow for an economical substitute for endoscopies (Hewetson, 2017). The use of a four-sugar absorption test will ultimately provide insight and better understanding of the permeability of the gastrointestinal tract and associated diseases. Sugar absorption tests would allow for an inexpensive and non-invasive model of gut permeability in the horse. The objective of the study was to measure the percent excretion of each of the sugars in pooled urine samples to determine the permeability of the gastrointestinal tract. To further the initial study, we focused on the correct mathematical equation to calculate percent excretion for each of the sugars detected in the urine samples.

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NonGlandular Grading System from the Equine Gastric Ulcer Council	
Grade 0	The epithelium is intact and there is no appearance of hyperaemia (reddening) or hyperkeratosis (yellow appearance to the squamous mucosa)
Grade 1	The mucosa is intact, but there are areas of reddening or hyperkeratosis (squamous)
Grade 2	Small, single, or multifocal lesions
Grade 3	Large, single, or multifocal lesions or extensive superficial lesions
Grade 4	Extensive lesions with areas of apparent deep ulceration

Figure 1: Scoring system for ulceration from Andrews 1999, Equine Gastric Ulcer Council.

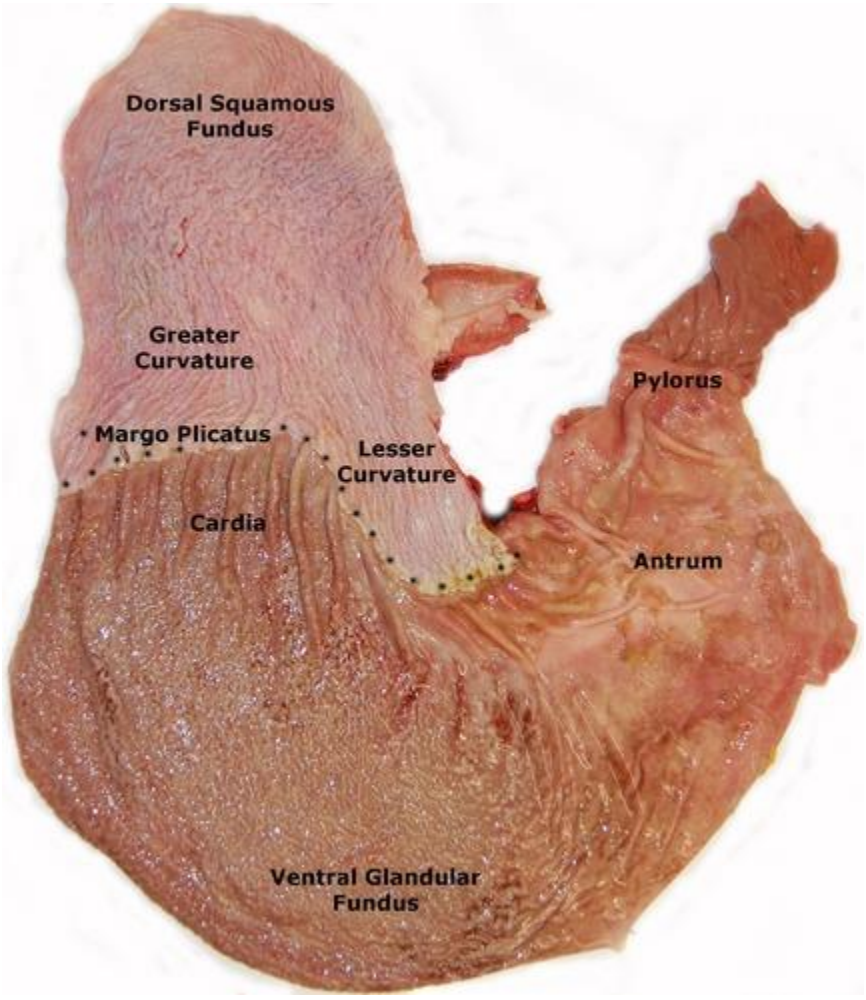

Figure 2: Equine Stomach Anatomy, from Sykes et. al., 2015

Chapter 2

Gastric Ulcers

2.1 Introduction

The increased concentrates fed to horses has increased gastrointestinal health issues; one of the most common being Equine Gastric Ulcer Syndrome (Sykes, 2015). Because of the numerous risks and causes of gastric ulcers in horses, it is hard to indicate a single solution. Endoscopy is one way to diagnose the severity of ulceration in the stomach. The Equine Gastric Ulcer Council created a scoring system that could be used to determine the severity (Andrews, 1999, Figure 1). This scoring system provides subjective descriptions of gastric ulceration in horses. Usually ulcers are being graded by individual veterinarians for clinical diagnosis, and less frequently the scoring system is being used as an outcome variable in research studies. In both of these circumstances it is important to better understand the inter and intragrader reliability that results. This knowledge could lead to improvements in the grading system or the training of individuals to use the grading system. Our hypothesis was that inter and intragrader reliability would be moderate or greater, that intragrader reliability would be greater than intergrader, and that while reliability may be moderate or greater, there may be measurable differences in the grades provided. Our objective was to evaluate reliability and distributions of grades produced from 5 graders grading 80 gastroscopies from horses with a wide range in gastric ulceration.

2.2 Materials and Methods

The increased concentrates fed to horses has increased gastrointestinal health issues; one of the most common being Equine Gastric Ulcer Syndrome. Because of the numerous risks and causes of gastric ulcers in horses, it is hard to indicate a single solution. Endoscopy is one way to diagnose the severity of ulceration in the stomach. The equine gastric ulcer council created a scoring system that could be used to determine the severity. This study used 80 endoscopy scores from five different graders to test the intra and inter grader reliability among scores for each of the regions of the stomach.

2.2.1. Study design

All procedures were approved by The Pennsylvania State University Institutional Animal Care and Use Committee (IACUC #46691). The study used eight Quarter Horse geldings that were subjected to four different dietary treatments using a Latin Square design. Four dietary treatments were 100% hay (HA), 40% hay and 55% whole oats (HO), 40% hay, 27.5% whole oats, and 27.5% pellets (HOP), and 40% hay and 55% pellets (HP). Two horses were on each diet every treatment period and each treatment was divided into four 14 day periods; each preceded by a 14 day hay only washout period. A final washout period of 14 days occurred before a 7-day ulcer induction period. The endoscopies were performed on the final day of the induction period. Following this period, the horses were turned out for 27 days on grass pasture to recover from the ulcers. On day 27 final endoscopies were performed to ensure horses were recovering from ulcers.

Endoscopies were performed on day 13 of every period in addition to day 7 of the ulcer induction. Horses were fasted between 16 and 20 hours, and water was withheld 4 hours prior to every endoscopy to ensure visibility of the stomach. Horses were sedated and restrained in stocks during each endoscopy, and all endoscopies were performed by veterinarians using techniques described in Murray (2002).

2.2.2. Endoscopy Videos:

A total of 80 endoscopy videos were collected throughout the study. To facilitate evaluation of intra-grader variation, 40 of the endoscopies were duplicated for a total of 120 videos of analysis. All 120 videos were scored separately by five graders who were blinded to the treatment groups. The Equine Gastric Ulcer Syndrome Council's grading system (Andrews et. al, 1999) was used to assess the non-glandular region. Descriptive terms adapted from Sykes et al. (2015), were used to describe the glandular region and can be found in Figure 3.

The non-glandular region was separated into four distinct scored regions: greater curvature (GC), lesser curvature (LC), dorsal squamous fundus (body), and esophageal orifice (EO). The EO region was removed from the scoring because it was poorly captured and ungraded in 24% of the videos. The EO represents the area immediately surrounding the esophagus just above the LC. Because of the unclear content in the EO we decided to pull these results from the analysis. The glandular region was divided into four graded regions: cardiac, fundus, antrum, and pylorus. Descriptive terms assigned to the glandular region were categorized as 0, 1, or 2, corresponding to sections that were unable to be seen (0), healthy (1), or annotated with some type of observation other than the former two categories (2). The scores from each

grader were then entered into a spreadsheet for each of the four glandular regions and four non-glandular regions.

Gastroscopies were graded by 5 graders. Graders 1 and 2 are veterinarians, 3 was an animal science graduate student, 4 has a PhD in animal science, and 5 is a veterinarian and expert on equine gastric ulcers. Graders 1-4 all received simultaneous training on the Equine Gastric Ulcer Syndrome Council's grading system (Andrews et al., 1999). Grader 5 was the primary author on the paper developing the grading system used.

2.2.3. Statistical Analysis

Intra and intergrader agreement was calculated for both the non-glandular and glandular regions. For the stratified squamous epithelium, reliability coefficients were calculated for each region using an unweighted and linear weighted agreement coefficient. The unweighted agreement coefficient assumes that there is no intermediate in a disagreement and treats all disagreements equally. It essentially gives zero weight to any disagreement between the graders (Graham, 1993). The weighted agreement coefficient however, measures the proportion of each grader's degree of agreement (Banerjee, 1999). The percent agreement as well as a 95% confidence interval, and the geometric mean were then calculated for each grader and region of the squamous mucosa.

The sum score used an intraclass correlation coefficient because it was a continuous variable and used the following grading scale: scores between 0.0 and 0.5 indicate poor

reliability, 0.5 and 0.75 indicate moderate reliability, 0.75 and 0.9 indicate good reliability, and those values greater than 0.9 indicate excellent reliability.

A weighted kappa statistic was used for the intergrader agreement because there are five unique graders with more than one rating (Wongpakaran, 2013). Intergrader reliability was assessed using Gwet's agreement coefficient with ordinal weights applied (AC_2) which allows for weights of partial agreement with the graders using ordinal data (Gwet, 2014). The benchmark scale was as follows: <0.0000 Poor, 0.0000-0.2000 Slight, 0.2000 – 0.4000 Fair, 0.4000-0.6000 Moderate, 0.6000-0.8000 Substantial, and 0.8000-1.0000 Almost perfect.

2.3. Results

2.3.1. Intragrader Reliability

The intragrader reliability estimates are reported in Table 1. The overall unweighted intragrader reliability was moderate to good and the weighted average was good to excellent. The difference between the unweighted and weighted indicates that while there some variability in scores, scores given to the same gastroscopies were usually within 1 grade of the other score given. There did not appear to be any clear trends of certain graders being more reliable than others. The lesser curvature had the lowest intragrader reliability, while the body had the highest. The high and sum score both had high reliability. Table 2 highlights that although the overall unweighted intragrader reliability was moderate to good and weighted average was good to excellent, there was statistical differences seen among the graders for each of the regions.

Figure 4 illustrates that there was a range in severity of ulceration over the 80 gastroscopies. There were predominately more grades between the 1 and 3 range, however there were grades of 0 and 4. The range in severity of ulceration shows that there was a variety of ulceration in the horses used for this study and the graders did in fact, find differences among gastroscopies they scored.

2.3.2 Intergrader Reliability

The intergrader reliability estimates are reported in Table 3. The overall weighted intergrader reliability for the greater curvature, lesser curvature, body, and high grade was substantial to almost perfect. There did not appear to be any trends of certain areas being more reliable than others. The body had the highest intergrader reliability, while the lesser curvature had the lowest.

2.4. Discussion

Analyzing intergrader and intragrader variability has been a common point of research not only in the field of equine gastric ulcers, but also in human medicine as well (Tammaa, 2015; Wise, 2020, Wongpakaran, 2013). Reliability is a measure of consistency. In this study reliability is how close were each of the scorers to one another, and to themselves, after using the stated scoring system. Intergrader reliability analyzes the variability between each of the five graders and intragrader reliability measures the variability within each of those five graders. The

agreement coefficient between and within graders is important on a clinical scale, especially if this grading scale is leading to diagnosis and treatment.

In this study, we investigated two characteristics of the ulcer grades that were assigned to the gastroscopy videos by the 5 graders. The first was reliability or agreement. This measure quantifies the degree of agreement between and within the graders and provides information on whether the score or grading can reliably be used by individuals and groups of graders. The second was whether there were statistical differences in the grades submitted by the different graders. This measure looks very precisely at whether there are differences between graders in the median score they provided. To understand these two characteristics, we can explore several hypothetical scenarios. In the first, the graders all provide the same grades as each other to each gastroscopy, resulting in perfect reliability and no difference in the grades between graders. In the second, the graders provide very different grades than each other to each gastroscopy, resulting in poor reliability and differences in the grades between graders. In the third, the grades provided are within one grade of each other for each gastroscopy, resulting in substantial reliability, but statistically measurable differences in the grades provided by each grader.

Our results fit within the third category. Our interpretation of this is that there was general agreement between the graders, but there were measurable differences in the grade they provided, even with that agreement. This may be because graders came from different background. The five graders in this study all had different backgrounds. Graders one and two were ambulatory veterinarians, three was an animal science graduate student, four has a PhD in animal science, and five is a clinical veterinarian and expert on equine gastric ulcers. The field of study of these individuals may be important to consider during our data analysis. Each grader went through proper training to grade the endoscopies for a subjective grading of the horse. The

ambulatory veterinarian graders tended to have higher ulcer scores compared to the equine researchers which may because each is focusing their attention on the severity for different reasons. A veterinarian has greater clinical experience than a scientist and hence a difference in scoring even after going through the same training. The intragrader agreement coefficients tended to be higher than that of the intergrader agreement coefficients may have to do with the background of each of the graders.

One study using children with limb fractures had their graders take measurements within two separate periods to estimate intragrader reliability after an elapsed period of time (Tammaa 2015). The graders in our study were blinded to dietary treatments and repeated gastroscopies. This could be another way to detect intragrader reliability and would be interesting for future studies to compare the results from both of these.

Our results did indicate that there was a statistical difference between the graders. Whether or not this had a direct correlation to their experience is uncertain, however the Wise 2020 study found that reliability was minimally influenced by experience. Both our results, and those from the Wise study, indicate that there is room for improvement within the grader scales being used (Wise, 2020). Improvement could be accomplished by increased training for each of the graders in order to increase the reliability.

2.5. Conclusion

The use of scoring system allows for uniformity among both researchers and veterinarians, however the reliability of the scores should be taken into consideration during analysis. This reliability has room for improvement by considering the clinical aspects of using

multiple graders and comparing findings across multiple studies. The background of each of the graders may play a role in their scoring, and increased training should be used with the scoring system.

2.5. Literature Cited

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Terminology and Ulcer Grading Sheet

Terminology and Ulcer Grading Sheet

Lesser Curvature - LC
Greater Curvature - GC
Body of Stratified Squamous Epithelium – aka dorsal squamous fundus, or the roof of the stomach
Esophageal Orifice – Area immediately surrounding the entrance of the esophagus into the stomach
Stratified Squamous Epithelium

Epithelium – The first layer of the mucosa which is exposed to the lumen

Mucosa – made up of three layers, of which the epithelium is the first layer

Hyperaemia – increased blood flow to the area

Hyperkeratosis – yellow appearance to the squamous epithelium

Glandular Region

Focal – Occurring in one particular site

Multi-focal – Occurring in many particular sites

Diffuse – Spread out throughout the entire glandular region

Mild/Moderate/Severe

Epithelial appearance (**hyperemic, hemorrhagic, fibrinosuppurative, ulcerated**)

Mucosal contour (**depressed, flat, raised**)

Lesion Grading System (1999 EGUS Council)	
Grade 0	The epithelium is intact and there is no appearance of hyperaemia (reddening) or hyperkeratosis (yellow appearance to the squamous mucosa)
Grade 1	The mucosa is intact, but there are areas of reddening or hyperkeratosis (squamous)
Grade 2	Small, single, or multifocal lesions
Grade 3	Large, single, or multifocal lesions or extensive superficial lesions
Grade 4	Extensive lesions with areas of apparent deep ulceration

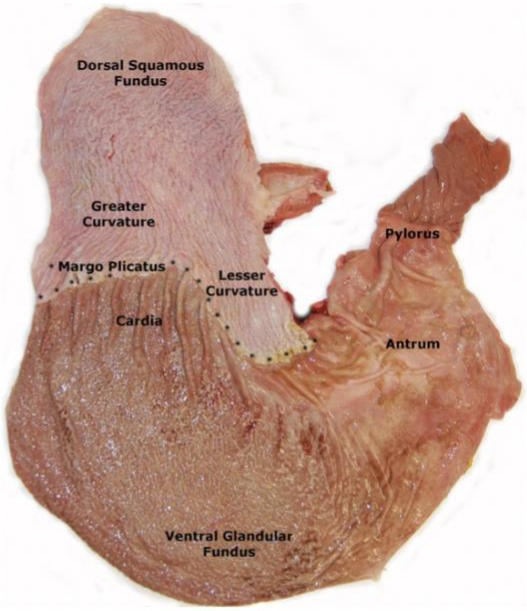

Figure 3: Terminology and Ulcer Grading Sheet

Table 1: Intragrader reliability estimates for 5 graders grading n=40 gastroscopies in duplicate

Grader		1	2	3	4	5	Geometric Mean
Greater curvature	Unweighted agreement (%)	55 (39-71)	75 (61-89)	70 (55-85)	80 (67-93)	68 (52-83)	69
	Unweighted AC	0.45 (0.25-0.66)	0.69 (0.52-0.87)	0.63 (0.45-0.82)	0.76 (0.60-0.92)	0.61 (0.43-0.79)	0.62
	Linear weighted agreement (%)	88 (84-93)	94 (90-97)	93 (89-96)	94 (89=98)	91 (87-96)	92
	Linear weighted AC	0.75 (0.64-0.86)	0.86 (0.78-0.94)	0.84 (0.76-0.92)	0.87 (0.77-0.96)	0.83 (0.74-0.91)	0.83
Lesser curvature	Unweighted agreement (%)	73 (58-87)	63 (47-78)	56 (40-73)	55 (39-71)	68 (52-83)	63
	Unweighted AC	0.67 (0.50-0.85)	0.54 (0.35-0.74)	0.47 (0.27-0.67)	0.46 (0.26-0.85)	0.60 (0.41-0.79)	0.54
	Linear weighted agreement (%)	92 (87-96)	89 (85-94)	88 (81-94)	87 (82-92)	89 (84-95)	89
	Linear weighted AC	0.84 (0.74-0.94)	0.77 (0.67-0.88)	0.74 (0.63-0.85)	0.73 (0.61-0.84)	0.76 (0.64-0.88)	0.77
Body	Unweighted agreement (%)	70 (55-85)	60 (44-76)	80 (67-93)	77 (63-91)	80 (67-93)	73
	Unweighted AC	0.65 (0.47-0.83)	0.52 (0.34-0.71)	0.77 (0.62-0.93)	0.74 (0.57-0.90)	0.78 (0.64-0.93)	0.68
	Linear weighted agreement (%)	92 (88-96)	88 (83-93)	95 (92-98)	94 (87-99)	95 (92-98)	93
	Linear weighted AC	0.86 (0.78-0.94)	0.77 (0.67-0.87)	0.92 (0.86-0.98)	0.90 (0.81-0.98)	0.94 (0.89-0.98)	0.88
High grade	Unweighted agreement (%)	70 (55-85)	60 (44-76)	63 (47-78)	68 (52-83)	73 (58-87)	67
	Unweighted AC	0.64 (0.46-0.82)	0.51 (0.31-0.71)	0.54 (0.35-0.73)	0.60 (0.42-0.79)	0.66 (0.48-0.84)	0.59
	Linear weighted agreement (%)	92 (88-96)	89 (85-94)	90 (86-94)	91 (86-95)	91 (86-96)	91
	Linear weighted AC	0.84 (0.74-0.92)	0.76 (0.66-0.87)	0.77 (0.68-0.87)	0.79 (0.68-0.90)	0.80 (0.68-0.91)	0.79
Sum	Intraclass correlation coefficient	0.83 (0.71-0.91)	0.85 (0.73-0.92)	0.84 (0.72-0.91)	0.78 (0.62-0.88)	0.81 (0.67-0.89)	0.82

Table 2: Geometric means and Kruskal Wallis tests of regional scores for each grader

Grader	1	2	3	4	5	Kruskal Wallis
Greater curvature	1.21 (1.02-1.41)[a]	1.09 (0.88-1.32)[a]	0.86 (0.68-1.06)[ab]	0.89 (0.70-1.09)[ab]	0.61 (0.44-0.80)[b]	20.6, 0.0004
Lesser curvature	1.56 (1.36-1.78)	1.05 (0.83-1.29)[a]	1.01 (0.82-1.23)[a]	0.76 (0.58-0.97)[a]	0.90 (0.69-1.14)[a]	24.6, 0.0001
Body	0.74 (0.59-0.91)	0.67 (0.50-0.86)	0.22 (0.12-0.33)[a]	0.25 (0.16-0.35)[a]	0.09 (0.02-0.17)[a]	81.7, 0.0001
High grade	1.81 (1.61-2.02)[a]	1.53 (1.29-1.79)[ab]	1.21 (0.99-1.46)[b]	1.13 (0.92-1.36)[b]	1.15 (0.91-1.42)[b]	18.8, 0.0008
Sum	3.69 (3.29-4.13)[a]	3.37 (2.91-3.90)[ab]	2.87 (2.51-3.29)[bc]	2.69 (2.34-3.09)[c]	2.63 (2.30-3.01)[c]	43.2, 0.0001

Table 3: Intergrader reliability estimates for 5 graders grading 80 gastroscopies.

Region		Greater curvature	Lesser curvature	Body	High grade
All Grader Scores	Unweighted agreement (%)	55 (49-61)	53 (48-58)	55 (49-62)	54 (47-60)
	Unweighted AC	0.46 (0.39-0.53)	0.43 (0.36-0.49)	0.49 (0.41-0.56)	0.43 (0.36-0.51)
	Linear weighted agreement (%)	87 (85-89)	85 (83-87)	86 (84-89)	85 (83=88)
	Linear weighted AC	0.72 (0.68-0.77)	0.67 (0.62-0.72)	0.77 (0.72-0.83)	0.68 (0.62-0.73)

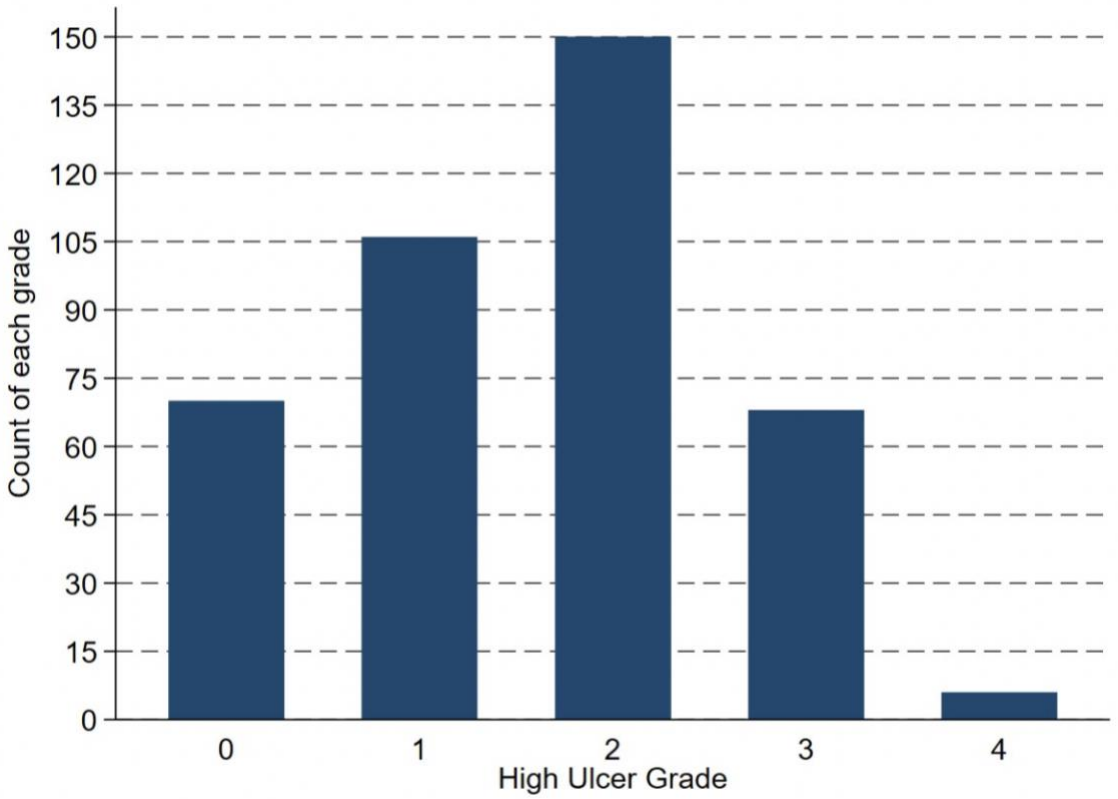

Figure 4: The figure illustrates the count of each grade when it was the highest score given for all regions of the stratified squamous epithelium for all the graders. This figure provides a view of the distribution of ulceration seen in the gastroscopies used for this study.

Chapter 3

Permeability

3.1. Introduction

The integrity of the gastrointestinal tract lining is critical to maintaining the health and wellbeing of animals. The protective barrier function of this bilipid membrane includes immune, secretory, and absorptive cells connected by tight junctions (Stewart, 2017). The tight junction proteins are capable of controlling permeability and when disrupted, larger molecules are able to pass through the lumen and can disrupt the homeostasis of the animal (Arrieta, 2006). Ulceration is a product of disruption to the barrier function of the epithelial cells (Lee, 1997). Utilizing a four-sugar absorption test will allow for detection of permeability along the gastrointestinal tract lining. Sucrose has been used in equine studies to detect the presence of gastric ulcers with limited success (Hewetson, 2006; O'Connor, 2004).

Our study focuses on the use of four sugars: lactulose, mannitol, sucrose, and sucralose. The percent excretion of these four sugars from pooled urine samples is used to determine permeability throughout the entire gastrointestinal tract. The samples are taken from two previous studies (Norris, 2013; Lapinskas, 2017). Further analysis of these samples made it clear that a correction factor was required for permeability calculations. This study focuses on the correct calculations and derivation of a new correction factor to calculate percent excretion for each of the sugars.

3.2. 2013 Study Materials and Methods

3.2.1. Treatments

The first study was conducted in 2013 by Kathryn Norris. The animals used in this study were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC #37483). Nine Quarter Horse yearlings, four females and five geldings, were utilized in a Latin Square design 84 days in length. The study consisted of three 28-day periods with a 14 day washout interval followed by a 14 day treatment interval. Horses were fed either a low concentrate high forage (LCHF) diet or a high concentrate low forage diet (HCLF) throughout the twelve-week period, alternating every 14 days. Within the HCLF treatment intervals horses were additionally supplemented with one of three top-dressed colostrum treatments; 70 g whole bovine colostrum (WBC), 70 g concentrated bovine colostrum (CBC), or no colostrum (NC).

On urine collection days, day 14 of each interval, horses were fasted for 16 hours prior to administration of sugar markers. Horses were brought into individual box stalls at 0530 h and remained in stalls until the following day at 0830 h to facilitate total 24-hour urine collection. Horses were not fed any concentrate meals on urine collection days. Horses were not allowed access to water from the hours of 0530 to 1000 h. For the reminder of the urine collections horses were allowed ad libitum access to water.

3.2.2. Sugar Absorption Tests

Total 24-hour urine collections were performed at the end of every 14-day interval. Horses were fasted for 16 hours prior to nasogastric administration of sugar markers. The sugar mixture contained 16.0 g mannitol, 28.0 g sucrose, 28.0 g lactulose, and 80.0 g sucralose dissolved and refrigerated in 500 mL of distilled water 12 to 24 hours prior to administration. Horses were fed a total of 12 kg hay throughout each 24-hour period, beginning 2 hours after sugar markers were administered and every four hours following. Horses were acclimated to urine collection devices prior to the collection periods. Horses wore urine collection devices for the entirety of the 24-hour period following sugar mixture administration to allow for total urine collection. Six-hour pooled urine samples (0 to 6hr, 7 to 12 hr, 13 to 18hr, and 19 to 24 hr) and a final total 24-hour urine sample were taken. These samples sat at ambient temperature in the barn over the 24-hour period. Aliquots were then frozen at -20℃ until further analysis.

3.2.3. Permeability Marker Analysis

Standards of sugar markers were dissolved in triple distilled water, aliquoted into 2.0 mL quantities, and frozen at -20℃. These were then used to validate consistent retention times, modify GC-run settings, and quantify standard curves. Details for GC validation are in Appendix A.

Urine samples and standards were thawed at room temperature and derivatized via an alditol acetate preparation for analysis via gas chromatography equipped with a flame ionization detector (GC-FID; procedure in Appendix B). The total run time was 42 min. All

chromatographic analysis was performed on an Agilent GC-FID 7890 (Agilent, Santa Clara, CA).

Area underneath the peak of each respective sugar was utilized to calculate the amount of sugar within the 100-microliter urine sample derived from a standard curve. This sugar weight was then adjusted to reflect total urine volume from that specific sample, providing total sugar excreted. Total sugar excreted was then divided by the weight of sugar administered at the start of the rest to provide the percent excretion of oral dose.

3.3. 2017 Study Materials and Methods

3.3.1. Horses

Eight Quarter Horse Geldings ranging from 3-15 years were used in the study and were housed in The Pennsylvania State University Almquist Research facility. Horses had continuous access to runs except during the 24h urine collections. All procedures were approved by The Pennsylvania State University Institutional Animal Care and Use Committee (IACUC #46691).

3.3.2. Experimental Design

Horses were fed four dietary treatments in a Latin Square design over a total period of 160 d. Two horses were on each diet every treatment period. Treatments were divided into four 14 d periods; each preceded by a 14 d hay only washout period. The last period was followed by

a 14d washout and a 7 d ulcer induction as a positive control. Endoscopies and 24 h sugar absorption tests (SATs) were conducted on d 13 during each washout and treatment period.

3.3.3. Treatments

Four dietary treatments were 100% hay (HA), 40% hay and 55% whole oats (HO), 40% hay, 27.5% whole oats, and 27.5% diet pellets (HOP), and 40% hay and 55% diet pellets (HP). To meet nutrient requirements HO, HP, and HOP diets had a balancer pellet (BP) added at 5%. Horses were fed treatment diets at 2% BW split into two meals, one fed in the morning and the second in the afternoon. Horses were transitioned to dietary treatments over the first two days of the treatment period.

3.3.4. Sugar Absorption Tests

Sugar absorption tests were performed immediately following endoscopies. Four sugars were administered in solution with distilled water at different dosages: mannitol (40 mg/kg BW), sucralose (200 mg/kg BW), lactulose (70 mg.kg BW) and sucrose (70 mg/kg BW). Horses were sedated based on demeanor and size to ease the nasogastric administration of sugar solution. Individual urinations were collected in urine collection devices. A 10% thymol in 100% isopropanol solution was added at 0.01 mL / mL of urine as a preservative. All urine was saved until the end of the 24 h collection. Subsamples of each urination, each 6 h pool (h 0-6, 7-12, 13-18, and 19-24 of the collection), and a total 24 h pool were collected and frozen at -20℃ until analysis. Blood was drawn at 0 min, 90 min, and 12 h in 7.0 mL silicone serum tubes (sBD

Vacutainer, Franklin Lakes, NJ) and centrifuged for 10 min at 500 g. Serum was aliquoted into 2 mL tubes and frozen at -20℃.

3.3.5 Permeability Marker Analysis

A previously validated alditol acetate derivatization process in conjunction with gas chromatography and flame ionization detection (GC-FID) was used to quantify sugar markers in urine. The details of this procedure are provided in Appendix B. An Agilent 7890A GC-FID was used for all chromatographic analysis. Individuals standards and a "stock" solution were used. Individual standards consisted of 25 mg of each sugar (mannitol, sucralose, lactulose, and sucrose) dissolved separately in 25 mL of double distilled water. The "stock" solution consisted of 25 mg of each sugar dissolved together in 25 mL of double distilled water. Standards were aliquoted and frozen at -20℃ until needed. Sugar standards were used to measure retention times, adjust GC run settings, and added to "spiked" samples.

3.4. Reanalysis

Using the 2013 and 2017 studies described above, we pooled both sample sets and created one spreadsheet. This spreadsheet contained the samples for both studies, the date the sample was ran, the horse the sample was collected from, the collection time, collection period, diet the horse was on, the spiked and non-spiked file name, the sugar weight, the inositol weight, the total urine volume, and the spiked sugar peaks and non-spiked sugar peaks, and then the

calculation of excretion for each of the sugars. Sample calculations can be found in Appendix A. After extensive analysis, it was determined that a correction factor was required to correct permeability calculations.

3.4.1. Correction Factor

The urine samples collected for this study have unknown quantities of each of the 4 sugar markers given in an oral dose to the horses. The quantity of sugar is determined by measuring the area under the curve of the sugar appearance in the GC detection. Two approaches can be used to quantify the amount of sugar based on this data. The first would be to compare the response to a standard curve of known sugar amounts added to a blank sample. The second approach is the one we used in our study. This approach is a standard addition method which uses the difference between the peak areas to determine the concentration of the unknown sample.

A correction factor was needed to correct for error in the urine collections, carrying out the assay procedure and running the samples through the GC. This could result from lack of precise measurements or multiple human and experimental factors. In this specific case, the correction factor corrects for the unknown amount of sugar present in each sample. Each urine sample contains an unknown amount of sugar with a known amount of each sugar marker added, so the correction factor (**CF**) is correcting for this unknown amount. After much discussion and review of the literature leading to the correction factor that we believe to be the best suited for our study, 3 different Correction Factor Calculations have been described below:

3.4.2. Comparing Rush, Sykes, and Our Equations

The RUSH Group correction factor is shown below. The basis of the calculation includes the spiked inositol peak area multiplied by the known amount added of the sugar (sucrose, mannitol, lactulose, or sucralose) divided by the peak area of the spiked sugar multiplied by the 0.02 mg of inositol. This correction factor was then multiplied by the peak area of the non-spiked sugar by the mg of inositol included. The RUSH Group correction factor also included a blank that was water with sugars added for their calculations, whereas our study used urine with sugars added as our blank for running standard curves. After analysis, we decided that using this correction factor was not appropriate for our analysis. The amount of sugar is not actually known in the 0.02 mg being added and this correction factor does not adjust for that amount of unknown. The correction factor accounts for the fact that different sugars will show up different on the GC, however it assumes that we know both the exact sugar and inositol being added, in which case we do not.

Rush Group:

$$\text{Correction Factor} = \frac{\text{Peak Area Spiked Inositol} \times \text{Weight of sugar spike added to sample}}{\text{Peak Area of spiked Sugar} \times \text{Weight of Inositol added to sample}}$$

The Sykes correction factor on the other hand, does consider that the sugar amount is unknown, and has an adjusted peak area. The adjust peak area subtracts the non-spiked peak area from the spiked peak area to account for the correct area difference.

Dr. Sykes PSU:

$$\text{Correction Factor} = \frac{\text{Peak Area Spiked Inositol} \times \text{Weight of sugar spike added to sample}}{\text{Adjusted Peak Area of Sugar} \times \text{Weight of Inositol added to sample}}$$

$$\text{Adjusted Peak Area} = \text{Spiked Peak Area} - \text{Non spiked Peak Area}$$

Reanalyzed Equation:

We decided the Rush correction factor would not be suitable for our data since we do not know the actual amount of sugar in the spiked tubes. We then discussed the use of the external standard curve method; however, we did not have enough standard curves for this to be accurate.

After much discussion with Hanna Van Every, we decided that the ratio of the AUC/g for inositol to the AUC/g of the sugar of interest is a property of the GC, the column, and the sugars, in which this ratio should not change if the GC and column don't change. Because we used a

spiked and non-spiked run in our calculations (which are two different samples), we need to account for extraction efficiency differences. This can be done by using the inositol data from both runs. The derivation can be outlined below:

Step 1: the efficiency factor is calculated to account for the differences in extraction efficiencies between the spiked and non-spiked samples

$$Efficiency\ Factor\ (EF) = \frac{\left[\frac{PA_{inositol}}{grams_{inositol}}\right]_{spiked}}{\left[\frac{PA_{inositol}}{grams_{inositol}}\right]_{nonspiked}}$$

Step 2: Using this efficiency factor, we can scale the non-spiked sugar peak areas. This derivation will continue to use mannitol as an example.

$$EF\ x\ [PA_{mannitol}]_{unspiked} = [PA_{mannitol}]_{unspiked,scaled}$$

Step 3: Now we have scaled the non-spiked sample, we can assume the extraction efficiency is the same in both the non-spiked and spiked samples.

$$\frac{\left[[PA_{mannitol}]_{spiked}\right]}{[grams_{mannitol}]_{spiked} + [grams_{mannitol}]_{unknown}} = \frac{[PA_{mannitol}]_{unspiked,scaled}}{[grams_{mannitol}]_{unknown}}$$

Step 4: This equation above states that the PA/grams for mannitol is constant, as long as the extraction efficiency is the same. Now that we have an unknown in our equation we need to isolate it which can be done by rearranging the equation. The first part of rearranging happens by multiplying both sides by $[grams_{mannitol}]_{unknown}$.

$$\frac{\left[[PA_{mannitol}]_{spiked}\right]x[grams_{mannitol}]_{unknown}}{[grams_{mannitol}]_{spiked} + [grams_{mannitol}]_{unknown}} = [PA_{mannitol}]_{unspiked,scaled}$$

Step 5: We can then move the entire bottom portion of the fraction by multiplying both sides by it to form the equation below.

$$\left[[PA_{mannitol}]_{spiked}\right]x[grams_{mannitol}]_{unknown} = \left[[grams_{mannitol}]_{spiked} + [grams_{mannitol}]_{unknown}\right]x[PA_{mannitol}]_{unspiked,scaled}$$

Step 6: Then using multiplication we can distribute $[[grams_{mannitol}]_{spiked} + [grams_{mannitol}]_{unknown}]$

$$\left[[PA_{mannitol}]_{spiked}\right]x[grams_{mannitol}]_{unknown} =$$

$$\left[[grams_{mannitol}]_{spiked}x[PA_{mannitol}]_{unspiked,scaled}\right] + \left[[grams_{mannitol}]_{unknown}x[PA_{mannitol}]_{unspiked,scaled}\right]$$

Step 7: We can then subtract this last term to the other side of the equation

$$\left[[PA_{mannitol}]_{spiked}x[grams_{mannitol}]_{unknown}\right] - \left[[grams_{mannitol}]_{unknown}x[PA_{mannitol}]_{scaled,unspiked}\right] = \left[\left[grams_{mannitol}\right]_{spiked}x[PA_{mannitol}]_{scaled,unspiked}\right]$$

Step 8: Now we can combine these terms

$$\left[[PA_{mannitol}]_{spiked} - [PA_{mannitol}]_{scaled,unspiked}\right]x[grams_{mannitol}]_{unknown} =$$

$$\left[\left[grams_{mannitol}\right]_{spiked}x[PA_{mannitol}]_{scaled,unspiked}\right]$$

Step 9: We can rearrange via division to isolate our unknown

$$\frac{[PA_{mannitol}]_{scaled,unspiked}\ x[grams_{mannitol}]_{spiked}}{[PA_{mannitol}]_{spiked} - [PA_{mannitol}]_{scaled,unspiked}} = [grams_{mannitol}]_{unknown}$$

3.4. Literature Cited

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Chapter 4

Summary and Thoughts

This thesis is a compilation of the undergraduate research work I have done throughout my time here at Penn State with Dr. Staniar. Although most of the work I have done and written about above has been furthering analyses from data already collected, there is more work to be done here. It should be noted that due to COVID-19 the research in both gastric ulcers and permeability was based on data that had been previously collected due changes in access to University research facilities and CDC guidelines. Before COVID-19 plans were in place to rerun samples in the laboratory, and to get new samples and data sets to further our studies. Below is a summary of my thoughts and implications on future studies for the gastrointestinal health of the horse overall, and specifically related to gastric ulcers and permeability in the gastrointestinal tract.

4.1 Gastric Ulcers

Gastric ulceration, EGUS, is a very prevalent issue in the equine industry today. Scoring systems have been developed to compare the severity of ulceration in the various regions of the stomach; however, the uniformity of these scoring systems is lacking. It was clear from our data that although the same scoring system was used for each grader, variability did exist. This variability is important to consider when comparing results across studies.

The graders of the study were as follows: two veterinarians, two were researchers and one was an ulcer expert. It was interesting to note that the researchers and veterinarians often had similar scorings even though all of the graders participated in taking the time to learn the terminology and how to correctly score ulceration based on the gastroscopies. Future studies may want to consider spending more time having each of the graders learn how to interpret the scoring system to reduce score variability. This has important implications when comparing the research side of the one-point difference between a score of a 1 or a 2, compared to the difference of a score of a 1 or 2 in the clinical field. A one-point difference in scoring means that the graders were different in their scoring and had variability, implying a need for a better, more unified scoring system. From a clinical perspective the one-point difference may not have as great of an effect and may lead to the same prognosis and treatment. This is an interesting area before further study to improve the scoring system of gastric ulceration in the horse.

4.2 Permeability

A four-sugar absorption test has never been used in the horse to determine gut permeability. Continued research in this area could have implications for a non-invasive, relatively inexpensive way of determining GIT health and function. The four sugars we have used; sucrose, lactulose, mannitol and sucralose, are all quantifiable in the urine and in plasma. While there are some complications to this method such as a rate of passage and how to correctly interpret the excretion percentage, future studies are required to confirm the capabilities of this test in determining barrier functionality in the horse.

The pools of urine samples were collected during different time periods with an overall 24 samples collected. Future research is needed to determine the best time period to analyze for percent excretion of the sugar. The proper correction factor for calculating the percent excretion of the sugars has been debated and will need further confirmation that our correction factor is being used properly based on our study.

While this study focused on urine samples, it would be interesting to repeat this study with serum samples instead and see if there are any differences and how they compare to one another. Future studies are needed to gather an increased sample size and confirm that this is a method that could potentially be used by horse owners, veterinarians, and researchers.

Appendix A

Sample Calculations

Knowns:

Spiked Mannitol = 884.8

Spiked Inositol = 2260.6

Non spiked mannitol = 288.9

Non spiked inositol = 2050.2

Weight of inositol = 0.1

Weight of sugar = 0.02

Total urine volume = 4365

Total sugar administration = 16000

Calculation 1: Rush Method

$$\text{Correction Factor} = \frac{\text{Peak Area Spiked Inositol} \times \text{Weight of sugar spike added to sample}}{\text{Peak Area of spiked Sugar} \times \text{Weight of Inositol added to sample}}$$

$$0.51 = \frac{(2260.6) \times (0.02\text{g})}{(884.8) \times (0.1\text{g})}$$

$$\text{Weight of sugar (mg) in 100µL urine} = \frac{\text{Correction Factor} \times \text{Peak area for non-spiked sugar} \times \text{Weight of inositol added to sample}}{\text{Peak area for non-spiked inositol}}$$

$$\text{0.0072 (mg) in 100µL urine} = \frac{0.51 \times 288.9 \times 0.1\text{g}}{2050.2}$$

$$\text{Sugar (mg) per mL of urine} = \text{Weight of sugar (mg) in 100µL urine} \times 10\text{ mL}$$

$$\text{0.072 (mg) per mL of urine} = \text{0.007 (mg) in 100µL urine} \times 10\text{ mL}$$

$$\text{Total urine sugar concentration (mg)} = \text{Sugar (mg) per mL of urine} \times \text{Total urine volume from respective collection (mL)}$$

$$\text{314.28 (mg)} = \text{0.072 (mg) per mL of urine} \times \text{4365 (mL)}$$

$$\text{\% Sugar excreted} = \frac{\text{Total urine sugar concentration (mg)}}{\text{Total sugar administration}}$$

$$1.96\% = \frac{314.28\ (\text{mg})}{16000}$$

Calculation 2: Sykes Method

$$\text{Adjusted Peak Area} = \text{Spiked Peak Area} - \text{Non spiked Peak Area}$$

$$595.9 = 884.8 - 288.9$$

$$\text{Correction Factor} = \frac{\text{Peak Area Spiked Inositol} \times \text{Weight of sugar spike added to sample}}{\text{Adjusted Peak Area of Sugar} \times \text{Weight of Inositol added to sample}}$$

$$0.76 = \frac{(2260.6) \times (0.02\text{g})}{(595.9) \times (0.1\text{g})}$$

$$\text{Weight of sugar (mg) in 100µL urine} = \frac{\text{Correction Factor} \times \text{Peak area for non-spiked sugar} \times \text{Weight of inositol added to sample}}{\text{Peak area for non-spiked inositol}}$$

$$\text{0.011 (mg) in 100µL urine} = \frac{0.76 \times 288.9 \times 0.1\text{g}}{2050.2}$$

$$\text{Sugar (mg) per mL of urine} = \text{Weight of sugar (mg) in 100µL urine} \times 10\text{ mL}$$

$$\text{0.11 (mg) per mL of urine} = \text{0.011 (mg) in 100µL urine} \times 10\text{ mL}$$

$$\text{Total urine sugar concentration (mg)} = \text{Sugar (mg) per mL of urine} \times \text{Total urine volume from respective collection (mL)}$$

$$\text{480.15 (mg)} = \text{0.11 (mg) per mL of urine} \times \text{4365 (mL)}$$

$$\text{\% Sugar excreted} = \frac{\text{Total urine sugar concentration (mg)}}{\text{Total sugar administration}}$$

$$3.00\ \% = \frac{480.15\ (\text{mg})}{16000}$$

Calculation 3: Reanalyzed Method

$$\text{Inositol Correction Factor (Efficiency Factor)} = \frac{\text{Peak Area Spiked Inositol} \times \text{Weight of Inositol added to sample}}{\text{Peak Area Non Spiked Inositol} \times \text{Weight of Inositol added to sample}}$$

$$1.10 = \frac{2260.6 \times 0.1}{2050.2 \times 0.1}$$

$$\text{Scaled, Non spiked Mannitol} = \text{Inositol CF} \times \text{Non spiked mannitol}$$

$$318.55 = 1.10 \times 288.9$$

$$\text{Weight of sugar (mg) in 100µL urine} = \frac{\text{Scaled, non spiked mannitol} \times \text{Weight of Sugar Added}}{\text{Spiked Mannitol – Non spiked, scaled mannitol}}$$

$$\text{0.011 (mg) in 100µL urine} = \frac{318.55 \times 0.02}{884.8 - 318.55}$$

$$\text{Sugar (mg) per mL of urine} = \text{Weight of sugar (mg) in 100µL urine} \times 10 \text{ mL}$$

$$\text{0.113 (mg) per mL of urine} = \text{0.011 (mg) in 100µL urine} \times 10 \text{ mL}$$

$$\text{Total urine sugar concentration (mg)} = \text{Sugar (mg) per mL of urine} \times \text{Total urine volume from respective collection (mL)}$$

$$491.12 \text{ (mg)} = \text{0.113 (mg) per mL of urine} \times 4365 \text{ (mL)}$$

$$\% \text{ Sugar excreted} = \frac{\text{Total urine sugar concentration (mg)}}{\text{Total sugar administration}}$$

$$3.07\ \% = \frac{491.12\ (\text{mg})}{16000}$$

% Excretion for 3 Methods:

Rush: 1.97%

Sykes: 3.00%

Ours: 3.07%

Appendix B

Assay Procedure

**Acknowledgements of protocol assistance to Katie Norris and Siga Lapinskas*

Reagents: Trifluoroacetic acid (TFA), Ammonium hydroxide, sodium borodeuteride, DMSO, glacial acetic acid, 1-methylimidazole, acetic anhydride, methylene chloride, acetone

Equipment: Heating block, blow-down apparatus, PYREX® 9mL Screw Cap Culture Tubes with PTFE Lined Phenolic Caps, 13x100mm (Product #9826-13), disposable glass Pasteur pipettes, 2.0ml amber GC vials, centrifuge, gas chromatograph

equipped for capillary columns and FID detector, a SPB-225 capillary column (Supelco) which was 30m×0.25mm I.D. column, with a 0.25μm film thickness.

*All reagents and materials purchased from Sigma Aldrich

All culture tubes and GC vials were labeled for sample identification that matched the laboratory book entry. 50μL of myo-inositol was added to every sample as an internal standard. Samples were run in duplicates – one non-spiked and one spiked sample. The spiked sample had 20μL of standard added in addition to myo-inositol. 100μL of urine is added to the appropriate culture tube following vortexing. The sample is dried under nitrogen. Once dry, 250μL of 2N TFA is added and the capped sample is heated for 60 min at 121°. After heating, the sample is dried under nitrogen. To facilitate complete evaporation of acid, 200μL of isopropanol is added, dried with nitrogen, and repeated for a total of two times. To reduce the sample, 100μL of 1M ammonium hydroxide is added. The sample is vortexed. 0.5mL of 20mg $NaBD_4$/1.0 mL DMSO solution (made earlier) is added and the sample is vortexed. The sample is then heated for 90 min at 40°. Following heating, 5-7 drops of acetic acid is added. Samples are vortexed. 100μL of 1-methylimidazole is added and samples vortexed. 0.5mL of acetic anhydride is added and samples vortexed. Let samples stand for 3-4 minutes until cool to the touch. 4mL of distilled water and 1mL of dichloromethane are added and samples are vortexed. Centrifuge sample at 500G for 1min to separate water and dichloromethane layers. The bottom dichloromethane layer is removed into a corresponding culture tube, “wash A”. 1mL of dichloromethane is again added to the initial culture tube, vortexed, and centrifuged, and removed into the “wash A” tube. 4mL of distilled water is added to the “wash A” tube, vortexed, centrifuged, and the dichloromethane layer is removed into the corresponding “wash B” tube. Once again 4mL of distilled water is added, vortexed, centrifuged, and the dichloromethane layer is removed to tube “wash C”. The 2mL of dichloromethane is then dried under nitrogen. 0.5mL of acetone is added to the sample and transferred to the appropriately labeled amber GC vial.

The detector temperature was 300°C and the injector temperature was 250°C. Initial column temperature was 100°C held for 2 minutes. Column temperature increased at a rate of 10°C/min held at 2 min. to 180°C and then at a rate of 4°C/min. to 210°C maintained for 32 min for a total run time of 51.5 min. An Agilent 7890A GC-FID was used for all chromatographic analysis.

Specific flow rates at the FID were 25mL/min of H_2, 35mL/min of N_2, and 300mL/min air. The inlet heater was 250°C, pressure at 7.8 psi, total flow was 24mL/min and the septum purge flow rate of 3mL/min. The inlet was set for a split ratio of 20:1.

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Specific retention time of each sugar (mannitol, sucralose, lactulose, sucrose, and inositol) are dependent upon each of the above listed details in addition to column length, deterioration of internal lining (based on how many samples have been analyzed), and peak size of compounds. Listed are initial retention times for each sugar. Note that retention times did shift over the analysis period, but the order of sugar peak appearance stayed the same: mannitol (39.009 min), sucralose (39.441 min), lactulose (41.188 min), sucrose (43.842 min) and inositol (46.354 min).

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ACADEMIC VITA

Education

- Pennsylvania State University - Schreyer Honors College — August 2017 - Present
  - Veterinary and Biomedical Science Major
  - Equine Science Minor

Work Experience

Veterinary Medical Center of Long Island - West Islip. NY

- Shadowed in the Operating Room, Physical Therapy, Exotics, Oncology Dentistry — June 2016
- Assistant to the Vet Tech in Emergency Room — July 2016 - 2018
- Worked part time during the months of June - August

Adventureland - Farmingdale, NY — March 2015 – 2019

- Ticket Booth Personnel / Group Sales Supervisor
- Ride Operator
- Concession Stand

Tutor Doctor — August – December 2019

- Tutor various subjects for high school and college students

Penn State University Math Department Proctor — August 2019 – 2020

- Proctor math exams for the department

Centre Animal Hospital

- Technician Assistant and Client Assistant — June 2020 – Present

Activities & Awards

Schreyer Honors College Student Council — August 2017 - Present

- Active Social Committee member
- Involved in Schreyer related events

Pre-Vet Club — August 2017 - Present

- Various Animal related service and education

Gamma Sigma Sigma - National Service Sorority — August 2017 - Present

- Treasurer - Fall 2019
- Financial Secretary – Spring 2020
- Corresponding Secretary – Fall 2020
- Parliamentarian – Spring 2021
- THON Finance Chair – 2018-2019
- Habitat for Humanity Service Trip
- Nittany Greyhounds
- Various Other Community Service Projects

THON

- Hospitality Committee — 2018-2019
- Finance Chair for Gamma Sigma Sigma — 2018-2019
- Alternative Fundraising Finance Captain — 2019-2020
- Independently Danced in THON 2020 — 2019-2020
- Corporate and Matching Checks Finance Captain — 2020-2021

Volunteer Activities

United Methodist Soup Kitchen - Farmingdale, NY — 2011 – Present

- Coordinating cooks and servers for the Soup Kitchen

- Serving and Assistant Leader

Habitat for Humanity — May 2018

- Spring Break Service Trip – New Smyrna, FL

Nittany Greyhounds

Hands on Therapeutic Riding — May-August 2019

Academic Awards & Achievements

- Schreyer Honors College — 2017

Research

- Lab with a focus on gastrointestinal health in horses — 2018 – Present