EFFECT OF DIETARY FIBER LEVEL ON THE

HEALING OF GASTRIC ULCERS IN HORSES

By

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Abstract: Equine Gastric Ulcer Syndrome (EGUS) is estimated to occur in 53 to 93% of performance horses. Feeding practices, management, and dietary composition have all been suggested to impact the frequency and severity of EGUS. The aim of this study was to determine if alteration of fiber content within the concentrate portion of the diet alters gastric ulcer scores. The effects on healing of a commercially available high fiber and low fiber feeds were compared. The experiment was conducted in two phases. During phase I, 21 mature Quarter Horses were managed under a 7 d feeding/fasting regimen to induce gastric ulceration. Horses were fed ad libitum grass hay for 24 hr, followed by 24 h fasting. After d 7, gastroscopic examination was performed to determine ulcer scores of the greater (GC) and lesser curvature (LC) of the stomach. Following gastroscopy, 16 horses with an ulcer score of ≥ 1 in GC and ≥ 2 in LC were selected for phase II. Horses were pair matched by weight, gastric ulcer score, and type and assigned to either the high fiber (HF) or low fiber group (LF) in a randomized block design. Each group was fed their respective ration for 30 d in 50:50 ratio with grass hay at 2% of BW in DMI. Gastroscopic examination was performed on d 0, d 15, and d 30 of phase II to evaluate the occurrence of gastric ulceration. During this time, a stomach fluid sample was also extracted from the gastric antrum of the stomach and pH was recorded immediately. Data were analyzed with Proc GLM (SAS) suitable for repeated measures. Gastric ulcer scores in both groups had improved from d 0 to 15 and d 15 to 30 ($P < 0.05$). The LF group had a greater stomach pH of 4.46 ($P < 0.05$) compared to 2.92 in the HF group on d 15. The results of this study suggest that an increase in fiber level in the concentrate portion of the diet does not alter the rate of gastric ulcer healing using an induced ulcer model.

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CHAPTER I

INTRODUCTION

The purpose of this study was to determine if alteration of fiber content within the concentrate portion of the diet alters gastric ulcer scores. More specifically, this trial was interested in the rate of healing of gastric ulcers in horses fed different concentrate diets.

Statement of the problem

Equine Gastric Ulcer Syndrome (EGUS) is found in approximately 53 to 93% or the horse population (Andrews et al., 2005). Many factors can alter the environment of the stomach and increase development of EGUS, such as stall confinement, time allowed on pasture, feeding schedule, diet, exercise, (Reese et al., 2009), stress (Blikslager and Wilson, 2019), transportation (Blikslager and Wilson, 2019) and nonsteroidal anti-inflammatory drugs (Andrews et al., 2005). Gastric ulcers impact overall gastrointestinal health, and may lead to secondary issues including lack of appetite, dull hair coat, decreased performance, and colic. With a high prevalence of EGUS in the horse industry, recent research has been directed towards additional management solutions aside from medicated treatment. Previous research has suggested that diet changes, such as forage type, could aid in healing of EGUS. However, comparison of the concentrate portion of the diet has not been evaluated in relation to EGUS healing.

Purpose of the study

The objectives of this study were:

1. Determine the effects of varying fiber levels in the diet on the healing of gastric ulcers in horses.

Hypothesis

The null hypothesis of this study is as follows:

There is no significant effect of fiber level in the diet on the rate of healing of gastric ulcers, stomach fluid pH, cortisol concentration, or complete blood count.

The alternative hypothesis of this study is as follows:

If the null hypothesis is rejected, then the effect of fiber level in the high fiber (HF) diet improved the rate of healing in gastric ulcers compared to the low fiber (LF) diet. In addition to this, fiber level effected stomach fluid pH, cortisol concentration, and complete blood count levels.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Equine Gastric Ulcer Syndrome (**EGUS**) is found in approximately 53 to 93% of the horse population (Andrews et al., 2005). Gastric ulcers are lesions, or ulcerations, commonly found throughout the glandular and nonglandular stomach, distal esophagus, and proximal duodenum (Flores et al., 2011). While ulcers occur throughout the digestive tract, they are found predominately in the stomach. Many factors can alter the environment of the stomach and increase development of EGUS, such as stall confinement, time allowed on pasture, feeding schedule, diet, exercise, (Reese et al., 2009), stress (Blikslager and Wilson, 2019), transportation (Blikslager and Wilson, 2019) and nonsteroidal anti-inflammatory drugs (Andrews et al., 2005).

Gastric ulcers impact overall gastrointestinal health, and may lead to secondary issues including lack of appetite, dull hair coat, decreased performance, and colic. While many horses are effected by EGUS, horses in race or show horse training programs are more susceptible with 91% of racehorses and 58% of performance horses being effected (Andrews et al., 2005). In a group of 365 horses varying in age, breed, and discipline

44% had evidence of gastric ulcer, with 44% having colonic ulceration as well (Franklin 2005). Franklin (2005) also reported a gastric ulcer incidence of 87% in a group of 180 show, race, and sport horses, with 63% experiencing colonic ulceration. This data shows a varying prevalence level in horses in different sectors of the industry.

Due to the prevalence of EGUS in horses additional management practices beyond medication for treatment and management are needed. While ULCERGARD® and GASTROGARD® (Merial, 2018) are effective in preventing and treating ulcers, some horse owners may find these options costly. Treatment costs may be as much as $8.25 to $16.50 per day with the preventative ULCERGARD® dependent on the size of the horse. Treatment of diagnosed ulceration with GASTROGARD® is recommended for a 28-day period with a cost of approximately $896. Depending on the frequency of product usage and prevention versus treatment, costs related to ulcer management can quickly become prohibitive.

Anatomy of the Stomach

Understanding the physiological design of the horse allows for a greater knowledge of gastrointestinal health and EGUS. The stomach is comprised of 4 anatomical regions: the cardiac, fundic, body, and pyloric. The tight curve of the lesser curvature positions the cardia and pyloric adjacent to each other (Blikslager and Wilson, 2019). The mucosal lining throughout the stomach contains distinct tissue types relative to the region of the stomach, including the pyloric mucosa, glandular mucosa, cardiac

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epithelium, and the nonglandular stratified squamous epithelium (Blikslager and Wilson, 2019). More generally, the upper portion of the stomach, is classified as a stratified squamous mucosa (nonglandular) and the lower portion containing a glandular mucosa (Reese et al., 2011).
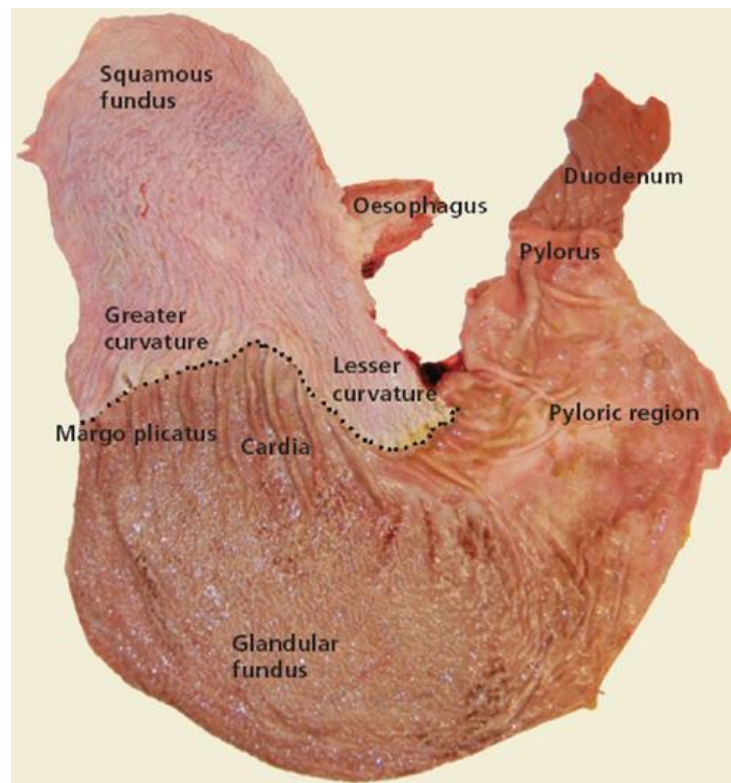
(Hepburn, 2011)

A large portion of the stomach is considered nonglandular (Blikslager and Wilson, 2019), with 80% of ulcers found in this portion of the stomach (Videla et al., 2009). The nonglandular portion of the stomach is composed of epithelial layers which are not primarily designed to be a barrier to acid diffusion since acidic fluid normally sits in the antrum of the stomach. Due to the lack of secretion, little is known about the true

function of the stratified squamous mucosa, but is speculated to provide protection to tissue layers from roughage (Blikslager and Wilson, 2019). Generally, gastric contents do not surpass the natural fill of the margo plicatus, preventing contact of acidic fluid with the squamous mucosa (Blikslager and Wilson, 2019), except during certain events like exercise (Reese, 2009). Without significant protective factors, when the stomach is in a state of low pH for long durations of time, this portion of the stomach is at greater risk of developing gastric ulcers.

The composition of the stomach lining changes at the margo plicatus, to cardiac glandular mucosa (Blikslager and Wilson, 2019). Differing from the upper region of the stomach, the glandular region produces hydrochloric acid, bicarbonate, pepsinogen, and mucus. The gastric mucosa contains parietal cells involved in the production of HCl, chief cells for pepsinogen production, and protective factors like bicarbonate (Blikslager and Wilson, 2019) which can impact stomach pH. Only 20% of gastric ulcers are found in this portion of the stomach (Videla et al., 2009). The lower occurrence of ulcers in the glandular region is largely due to the protective components mucus and bicarbonate provide. Ulcers in this region are usually developed due to stress-induced release of cortisol and the use of nonsteroidal anti-inflammatory agents, which are typically responsible for degrading stomach barrier function. (Andrews et al., 2005).

Stomach pH

Stomach pH has been identified as one of the factors which can damage the stomach lining and cause ulceration. Ulceration is caused from the swelling of stomach

cells from acid damage, due to volatile fatty acids (**VFAs**) and lactic acid passing into the

squamous mucosa when pH is 4 or less (Zavoshti, 2017). It has been suggested that

factors aside from hydrochloric acid (**HCl**) secretion could contribute to gastric

ulceration in the stratified squamous mucosa, as this region of the stomach is fairly

impermeable (Blikslager and Wilson, 2019). Depending on the area of the stomach

measured, pH ranges from 1.5-7.0 (Andrews and Nadeau, 1999). In the glandular portion

of the stomach, the average pH is 3.0 over a 24 h period (Sykes and Jokisalo, 2015;

Campbell-Thompson and Merritt, 1987).

There are other important physiological aspects of the equine stomach. Horses

are designed to be continuous grazers, due to a low capacity, simple stomach (Reese et

al., 2009). The stomach only makes up 8-10% of the gastrointestinal tract, with the ability

to hold 7.5-15 L (Jassim and Andrews, 2009). As a result of this anatomical design, the

stomach continuously produces HCl independent of feed being present (Flores et al.,

2011). The relatively low pH, compared to values of 5-7 in cattle (Grunberg and

Constable, 2009) and constant exposure to HCl is thought to be a major contributing

factor to EGUS.

The presence of feed can increase the production of additional hormones such as

gastrin which stimulates histamine, leading to increased HCl production (Blikslager and

Wilson, 2019). Lower pH is essential for protein digestion through activation of the

zymogen pepsinogen. Certain areas, such as the nonglandular region of the stomach,

however, can be damaged by HCl and pepsinogen secretion (Blikslager and Wilson,

2019). Under normal conditions hydrochloric acid is secreted in the gastric mucosa which maintains a high transepithelial electrical resistance to inhibit backflow of H+ ions. This action of protection is one of the many mechanisms present to avoid acid damage (Blikslager and Wilson, 2019). A gel with HCO3- is formed by mucus, which also plays a role in protection as it neutralizes acid before moving into the lumen (Blikslager and Wilson, 2019). The ability of the stomach mucosa to maintain proper defense against acid damage is crucial to prevent ulceration (Blikslager and Wilson, 2019).

Pathophysiology of ulcer development

Various aggressive and protective factors are found in the stomach that contribute to digestion and maintain the integrity of the stomach lining. Aggressive factors are commonly seen as factors in the stomach that could cause inflammation or irritation. These include HCl, organic acids, pepsinogen (converted to pepsin), and the duodenal reflux of bile acids (Sykes and Jakisalo, 2015; Rabuffo et al., 2009). The stomach endogenously produces hydrochloric acid, which has been recognized to be the most prominent aggressive factor (Sykes and Jokisalo, 2015; Berschneider et al., 1999), although critical for protein digestion. Nonsteroidal anti-inflammatory drugs have also been noted to impair the natural protective mechanisms of the stomach, especially prostaglandin secretion through inhibition of cyclooxygenase 1 and 2 (Blikslager and Wilson, 2019). This disruption decreases mucosal blood flow in addition to mucus and bicarbonate production, which may contribute to ulceration in the gastric mucosa (Blikslager and Wilson, 2019).

Other protective factors include epithelial restoration and mucosal blood flow, provided by the nonglandular and glandular mucosa (Sykes and Jakisalo, 2015; Rabuffo et al. 2009). Bicarbonate and prostaglandin E are additional protective factors supplied by the glandular mucosa (Sykes and Jakisalo, 2015; Rabuffo et al. 2009). However, the rate at which these factors are secreted or present is not always in balance. Therefore, ulceration has been attributed to differences in the levels of protective and aggressive factors (Blikslager and Wilson, 2019).

While the stratified squamous epithelium has the capability to be resistant to HCl damage, substances such as bile salts and short-chain fatty acids have been found to break down this barrier in low pH (Blikslager and Wilson, 2019). Consequently, layers of the epithelium that are not typically exposed to HCl can be affected and more susceptible to ulceration (Blikslager and Wilson, 2019). This study also reported that factors such as feed deprivation and intensive training can have a greater impact on the development of squamous epithelial ulceration with prolonged exposure to HCl.

Damage to the gastric mucosa may be caused by different factors than to the stratified squamous mucosa (Blikslager and Wilson, 2019). Infection of *Helicobacter pylori* is the cause of most ulceration found in the gastric mucosa in humans (Blikslager and Wilson, 2019). *Helicobactor pylori* cause inflammation within the stomach (Blikslager and Wilson, 2019). It has been speculated that acid tolerant bacteria such as Lactobacillus, Streptococcus, and Escherichia coli may lead to further damaging ulcers (Zavoshti and Andrews, 2017). Helicobacter DNA has been found in the stomach mucosa

in the horse, but it hasn't been determined if bacterial presence is a key contributing

factor of ulceration in horses.

Equine gastric ulcer syndrome (EGUS)

Management practices can increase the risk for a healthy horse to develop EGUS as a

primary disease (Sykes and Jokisalo, 2015), with several factors influencing the level of

risk of EGUS. These include stall confinement, being on pasture, feeding schedule, diet,

exercise, (Reese et al., 2009), stress, transportation (Blikslager and Wilson, 2019) and

nonsteroidal anti-inflammatory drugs (Andrews et al., 2005). Zavashiti and Andrews

(2017) reported that rigorous management practices that include many of these risk

factors, make a digestively healthy horse more susceptible to HCl and organic acid

exposure.

Various studies contribute squamous ulceration to over exposure of HCl, VFAs,

and lactic acid production from resident stomach bacteria (Zavashiti and Andrews, 2017).

The squamous fundus lacks sufficient protective ability during constant exposure of

extremely acidic gastric fluid, resulting in the formation of squamous ulceration (Sykes

and Jokisalo , 2015; Lorenzo-Figueras and Merritt 2002). A mucosal barrier is present in

the squamous mucosa, but it does not provide a substantial mucus and bicarbonate layer

and has a poor blood supply (Zavashti and Andrews, 2017). The presence or lack of these

protective and aggressive factors, which can be impacted by management practices,

influence a horse's risk for EGUS.

Clinical signs and diagnosis

Poor body condition, weight loss, diarrhea, changes in behavior, lack of appetite and poor performance are vague clinical signs of ulcers (Zavashti and Andrews, 2017; Camacho-Luna et al., 2018). Colic, specifically recurring colic, is one of the most frequent clinic signs of EGUS, even though not all horses that have ulcers show symptoms of colic (Zavashiti and Andrews, 2017; Camacho-Luna et al., 2018). Nonspecific signs for EGUS, such as pain and discomfort when tightening the cinch/girth have been observed, as well as stereotypic behaviors like cribbing, head nodding, wall kicking, pawing, wood chewing, and weaving in the stall (Zavashti and Andrews, 2017; Camacho-Luna et al., 2018). However, some studies have failed to show a relationship between the presence of gastric ulcers and crib-biting or weaving (Camacho-Luna et al., 2018).

Poor performance is significantly concerning to many horse owners, but the relationship between performance success and EGUS has not been extensively studied (Zavashti and Andrews, 2017). Clinical signs are sometimes not apparent even when gastric ulcers are present. Yet, when clinical signs are present, gastric ulcers are typically more severe when compared to horses that do not show clinical signs (Andrews and Nadeau, 1999). It should still be noted, though, that clinical signs do not always associate with the severity and occurrence of ulcers (Camacho-Luna et al., 2018). In addition to this, these vague clinical signs and changes in behavior could be attributed to other environmental factors such as training.

Currently, the only accurate form of diagnosis of gastric ulcers is gastroscopic examination. During this procedure, many veterinarians use a scoring system (MacAllister et al., 1997) that assigns a score of 0-5 to assess ulcer severity with 0 indication no lesion and 5 indicating an ulcer with active hemorrhage. While this procedure requires light sedation of the horse, it is minimally invasive. Endoscopy can be costly to horse owners and requires the horse travel to an equine clinic to be evaluated. Alternative diagnostic methodology such as evaluating sucrose permeability of urine and guaiac-based fecal occult blood tests have shown inconsistent results in their accuracy of the presence and location (stomach or colon) of ulcers (Zavoshti and Andrews, 2017). Further research is still necessary to identify other accurate methods of diagnosing EGUS aside from gastroscopy.

Housing

Differences in gastric ulcer prevalence has been observed in pastured versus stalled horses. Horses continuously grazing pasture have more buffering capacity and a higher stomach pH due to a more continuous supply of saliva (Andrews et al., 2005). Horses consistently kept in stalls are typically at a greater risk of gastric ulcers, due to restriction from active grazing for a large portion of the day. Without continuous grazing or forage consumption, not only is there a lack of continuous saliva to help buffer the stomach, but the gastric environment will also become more acidic (Reese et al., 2009). As a result, the stomach remains at a lower pH for longer durations of time thus putting the stomach at risk for damage and irritation. Allowing more continuous access to pasture

with adequate forage will not entirely eliminate a horse's susceptibility if other

contributing factors are present, but could help reduce the incidence of gastric ulcers.

Exercise

Advanced exercise routines alter the abdominal cavity and can initiate changes in

gastric health in performance and race horses. Heavily exercised horses have shown the

highest incidence of EGUS; with 70% or greater of Thoroughbred racehorses affected

(Sykes and Jokisalo, 2015), 93% of competing endurance horses (Tamzali et al., 2011)

and 63-87% of Standardbred racehorses affected by ESGUS (Rabuffo et al., 2002; Sykes

and Jokisalo, 2015). Show horses have also been affected by EGUS at 58% (McClure et

al., 1999).

Intense exercise has a strong correlation to the incidence of EGUS, with risk of

development rising as intensity of exercise increases (Sykes and Jokisalo, 2015). Exercise

is believed to influence EGUS as during galloping there is an increase in abdominal

pressure and decrease in stomach volume which can push stomach acid towards the

nonglandular portion of the stomach (Reese et al., 2009). It has also been observed that

horses in training have a higher level of serum gastrin concentration which can lead to an

increase in HCl concentration and lower pH (Andrews et al., 2005).

Feeding schedule

Feeding schedule has been identified as another contributing factor to the

prevalence of gastric ulcers in horses. Zavoshti and Andrews (2017) recommend feeding

smaller, more frequent grain meals to reduce the amount of intragastric fermentation and decreases volatile fatty acid production. In a study by Bass et al. (2018), thirty-one 2-3 year old Quarter Horses were managed in a training program similar to what is seen in the stock type industry relative to western disciplines. These horses were fed 2% of their body weight in grass hay and a high fat/low starch grain. The traditional fed (TF) group was given grain in two feedings per day. The fractioned fed (FF) group had controlled automated feeders which provided a portion of grain every hour over 20 consecutive hours (Bass et al., 2018). No difference in ulcer scores was seen in the FF group at day 30 and 60 compared to day 0. However, a difference in ulcer scores at d 30 and 60 was significant in the TF group (Bass et al., 2018). Bass (et al., 2018) reports that extended fasting periods seen with feeding twice per day could be a factor for ulcer development.

In addition to feeding the grain portion of the diet in smaller portions, increased forage intake has been shown to help reduce the prevalence of gastric ulcers as well. Allowing horses to be out on pasture with ample forage, instead of stalls or dry lots, can improve this condition and reduce the likelihood of ulcers developing. Horses continuously grazing on pasture have more buffering capacity and a higher stomach pH due to a more continuous supply of saliva (Andrews et al., 2005). Horses consistently kept in stalls are typically at a greater risk of gastric ulcers, with the horse being restricted from active grazing for a large portion of the day. Without continuous grazing or forage consumption, not only is there a lack of continuous saliva to help buffer the stomach, but the gastric environment will also become more acidic (Reese et al., 2009). As a result, the

stomach remains at a lower pH throughout the day thus putting the stomach at risk for damage and irritation. Allowing more continuous access to pasture with adequate forage will not entirely eliminate a horse's susceptibility, but could help reduce the incidence of gastric ulcers.

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used in horses to treat inflammation and pain in soft tissue, the musculoskeletal system, and in the abdomen (Moses and Bertone, 2002; Kynch, 2017). A cascade of events take place at a cellular level following cellular injury, which causes inflammation (Moses and Bertone, 2002). Inflammation is beneficial to a certain degree, being viewed as an important piece of the healing process (Moses and Bertone, 2002). Substantial inflammation is typically due to the accumulation of prostaglandins (Moses and Bertone, 2002). Prostaglandin E_2 (PGE_2) in particular stimulates vasodilation, producing heat and superficial reddening of the skin (Moses and Bertone, 2002).

Common NSAIDs used in large animals inhibit the enzyme cyclooxygenase, reducing the conversion of arachidonic acid to prostaglandins and thromboxane (Moses and Bertone, 2002). This mechanism helps create an anti-inflammatory response by reducing redness, heat, swelling and pain (Moses and Bertone, 2002). There are two isoforms of cyclooxygenase, known as COX-1 and COX-2 (Moses and Bertone, 2002). Expression of these enzymes is seen extensively in the gastrointestinal tract (Kynch,

2017). Cyclooxygenase 1 is produced at a constant level in the body and is involved in maintaining gastrointestinal mucosal integrity, platelet aggregation, and renal blood flow (Moses and Bertone, 2002). Cyclooxygenase 1 aids in regulation of production of PGE_2 and other prostaglandins (Kynch, 2017). These prostaglandins play an important role in stimulating factors like bicarbonate and mucus, in addition to reducing the level of hydrochloric acid that is produced (Kynch, 2017). Prostaglandin metabolism equips the stomach with protective factors to prevent gastric acid injury. Inflammation does not stimulate COX-1 activity, but it could be involved in an inflammatory response (Moses and Bertone, 2002). Cyclooxygenase 2 is the inducible isoform involved in inflammation, mitogenesis, and specialized signal transduction (Moses and Bertone, 2002). Cytokines and bacterial lipopolysaccharides are involved in inflammation and induce COX-2 activity (Moses and Bertone, 2002).

Phenylbutazone and flunixin meglumine (Banamine ®) are among the most common FDA approved NSAIDs used in horses, being the primary source of treatment for pain and inflammation (Kynch, 2017). While this treatment is effective, it has been reported to cause gastric ulceration in cases of overdosing, prolonged administration, or vulnerable populations (Kynch, 2017). This gastric damage is linked to inflammation and decreasing protective prostaglandins (Kynch, 2017). These common NSAIDs are nonselective COX inhibitors, meaning they have potential to inhibit both COX-1 and COX-2 (Moses and Bertone, 2002). This mechanism can act on COX-2, reducing inflammation in the body. However, as a consequence of a nonselective NSAID, COX-1

reduction will disrupt the ability to maintain gastrointestinal protective factors. Due to the negative effects this class of NSAIDs can have on the health of the gastrointestinal tract, it has been recommended that COX-2 specific NSAIDs be utilized more commonly to avoid inhibiting the function of COX-1 (Kynch, 2017).

Stress

Studies have been conducted to evaluate the impacts of stress in multiple livestock species. However, the mechanism of stress impacts multiple body systems (i.e. reproductive system, gastrointestinal system), making it difficult to isolate factors that initiate the stress response. A relationship between stress and gastric ulceration was suggested by Hans Selye in 1943 with a study involving rodents (Scheidegger et al., 2017). Communication pathways shared between the brain and gastrointestinal tract take place through the various nervous systems, including the central, enteric, and autonomic nervous system in addition to the HPA axis (Koneurek et al., 2011). Upon the discovery of this relationship, researchers have further investigated the impacts of stress and gastric ulcers in horses.

Malmkvist (et al., 2012) conducted a study to evaluate behavior and stress response in horses with gastric ulceration. Sixty Danish warmbloods between 3 and 19 years of age were kept in individual stalls. Horses were grouped based on the presence of gastric ulceration. Thirty horses were classified as an ulcer group with gastric ulcer scores of 3 and 4 found in the glandular mucosa. Another set of thirty horses was classified as the control group with an intact mucosa, having ulcer scores of 0 or 1. In this group with

an ulcer score of 1, the mucosa had no ulceration but could show reddening or hyperkeratosis. Of the horses that had an ulcer score of 2-4, 40.6% of them showed ulceration in the non-glandular squamous region of the stomach whereas 55.2% had ulceration in the glandular portion. Malmkvist (et al., 2012) used a novel object test with movement of a traffic cone for 10 minutes to create a stressor and evaluate the horse's behavioral response in addition to cortisol levels during times of stress. Fecal cortisol metabolites (FCM) indicate levels of blood cortisol with a 24 h lag time. A basal FCM was collected before the novel object test was conducted and 24 h after the novel object test. Both groups in this study did not show a difference in basal cortisol levels. However, horses in the ulcer group had a 26% higher concentration of FCM following the novel test when compared to the control group.

Malmkvist (et al., 2012) did not distinguish whether horses with higher FCM are in a state of stress more often, leading to higher cortisol level, or if they simply respond in different ways to various acute stressors. It was difficult to conclude if higher FCM levels were a consequence of the presence of gastric ulceration or if more stressed horses were simply more susceptible to ulcers. Others have proposed that a higher level of cortisol reduces the ability for the glandular mucosa to maintain its protective ability, thus creating a higher risk of ulcer development.

Scheidegger (et al., 2017) also discusses how understanding the pathophysiology of stress can be difficult to discern. Scheidegger (et al., 2017) evaluated the response of 26 sport horses with gastric ulcers to an adrenocorticotropic hormone (ACTH)

stimulation test. In this study, horses with more severe ulceration had a stronger response to the ACTH test with elevated salivary cortisol concentration. This indicated that there is a relationship present between stress-sensitivity and gastric ulceration. Scheidegger, like Malmkvist, found it difficult to determine whether horses with gastric ulceration were naturally hypersensitive, if adrenocortical hypersensitivity was an adaptive mechanism of the hypothalamic pituitary adrenal axis, or if multiple factors attributed to these results. It was also speculated as to whether inflammation and distress caused by ulceration naturally activated an increased adrenal response on its own.

Bacteria

Helicobacter pylori (H. pylori) has been attributed to gastric ulceration in other species such as mice and humans (Contreras et al., 2007). However, *H. pylori* has not been identified as a contributor to ulcers in horses (Camacho-Luna et al., 2018). In a study conducted by Contreras (et al., 2007), 20 Thoroughbred racehorses ages 2-6 were euthanized due to injury and stomach samples taken from the squamous and glandular mucosa. Of the sample group, horses with either ulcers, gastritis, or both, *Helicobacter*-like DNA was identified. However, *H. pylori* was not specifically identified in these horses. Regardless, in 90% of horses in this study, *Helicobacter* was present. Contreras (et al., 2007) speculates that *Helicobacter* species are found to some degree in the stomach independent of ulcers, gastritis, or normal mucosa.

It has been speculated that acid-tolerant bacteria such as *Escherichia coli, Lactobacillus,* and *Streptococcus* found in the stomach may increase the severity of

ulcers (Zavoshti and Andrews, 2017). Research regarding the presence of *H. pylori* and other bacteria in horses with gastric ulcers or normal mucosa is limited and relatively new area of interest due to its known relationship with ulcers in other species.

Diet

Performance horses are commonly fed high concentrate diets in order to meet higher energy requirements. This portion of the diet is composed of highly hydrolysable carbohydrates which are further processed into volatile fatty acids (Reese et al., 2009). In a low pH environment, these volatile fatty acids are more capable of entering the squamous mucosa and causing cell swelling, inflammation, acidification, and ulceration (Andrews and Nadeau, 1999). It is also important to be aware that horses have a lower pH during 1:00-9:00 AM regardless of environment type, which is typically during the time when the first feeding would be given (Reese et al., 2009). This, in conjunction to the biological breakdown of feed, could relate to why intermittent fed horses show more severe gastric ulcers (Reese et al., 2009). Reese and Andrews (2009) and Andrews and Nadeau (1999) reported that diets high in concentrates may lead to increased production of short-chain fatty acids which may increase the chance of gastric ulcers in the horse. Some may suggest reducing the concentrate portion of diet, however this isn't always a viable option to meet all of the nutritional requirements of a working horse.

Concentrate diets or supplements high in fat, such as corn oil, have also been observed to reduce acidity in the stomach. Reese and Andrews (2009) reported that

ponies fed corn oil daily had reduced gastric acidity and a higher concentration of prostaglandin. Therefore, it is speculated that fats could provide protective mechanisms to potentially help prevent ulceration (Videla and Andrews, 2009). However, other studies have reported no improvement in gastric ulcers with corn oil in the diet (Frank et al., 2005). This study, however, utilized a gastric ulcer induction protocol instead of using horses with naturally occurring ulcers.

Diets containing certain fiber sources in addition to higher levels of fiber may be expected to have a lower level of acid production and be less likely to cause ulcers (Lybbert et al., 2007; Nadeau et al., 2000). With the presence of forage in the stomach, more buffering capacity is available to counteract the acidity of gastric juices, resulting in an average pH of 7.0 at the cardia of the stomach (Sykes and Jokisalo, 2015; Husted et al., 2008). While different fiber sources such as alfalfa, Bermuda grass, and bromegrass hay have been compared (Lybbert et al., 2007; Nadeau et al., 2000), different fiber levels have not been extensively evaluated in a research setting in horses.

Treatment and prevention for EGUS

Due to a low rate of spontaneous healing, pharmacological treatment and nutritional management changes have been recommended to promote healing, especially in horses kept in training (Zavoshti and Andrews, 2017; Camacho-Luna et al., 2018). Removing a horse from environmental risk factors may reduce the severity of gastric ulcers, but may not result in healing. If ulcers are severe enough, pharmaceutical

treatment could be required to re-establish a typical appetite. This would likely help promote more roughage intake and aid in a more neutral pH in the stomach, supporting gastric healing (Sykes and Jokisalo, 2015). Other risk factors such as exercise are typically not going to be altered in the case of performance horses. Therefore, these horses will remain more at risk of EGUS, requiring other treatment solutions.

Reese and Andrews (2009) suggest that following pharmacological treatment, nutritional and dietary management changes should be implemented to reduce the chance of ulcers reoccurring. Other recommendations such as reducing the amount of exercise, allowing more time on pasture, increasing number of feedings, providing more forage and/or fiber, and decreasing the amount of nonstructural carbohydrates in the diet have been suggested to help reduce the prevalence of gastric ulcers (Camacho-Luna et al., 2018).

Omeprazole

Today, one of the most common forms of ulcer prevention and management is the use of omeprazole paste, with product names ULCERGARD® and GASTROGARD® (Merial, 2018). Omeprazole is a substituted benzimidazole compound which blocks the hydrogen and potassium ATPase enzyme system in the parietal cells of the stomach. (MacAllister et al., 1999; Zavoshti and Andrews, 2017). This enzyme is responsible for substitution of hydrogen and potassium ions to complete the production of HCl (Zavoshti and Andrews, 2017). Therefore, suppressing this enzyme inhibits gastric acid production (MacAllister et al., 1999).

Research conducted by MacAllister (1999) reviewed the efficacy of omeprazole paste to promote healing of gastric ulcers. The trial contained 140 horses of multiple breeds and sexes, ranging from 4 weeks to 28 years of age. Horse's rations and exercise regimen varied amongst the group due to specificity of breed type, condition, and age. Gastroscopy was performed to initially determine ulcer severity in the nonglandular mucosal region of the stomach and again at the end of the trial. Horses were divided into two groups to either receive a sham dosed control syringe or omeprazole paste. Each horse given omeprazole paste at 4 mg/kg of body weight. At conclusion of the trial, improvement in ulcer score was seen in 99% of omeprazole-treated horses, with 86.7% showing complete healing (MacAllister et al., 1998).

Vatistas (1999) evaluated the efficacy of omeprazole in healing gastric ulcers in horses maintained in race training. For 28 days, 14 Thoroughbred horses in active race training were put on trial for 28 d. The horses were divided into two groups, where 7 horses were given 1.54 g/d of active omeprazole and 7 horses received a placebo paste. The horses were endoscopically examined at the beginning of the trial, at 13-17 days, and 27-31 days. When comparing the two groups, horses treated with omeprazole paste showed a significant reduction in the severity of ulcers at 13-17 days and 27-31 days (Vatistas et al., 1999).

Nutrition

While omeprazole is effective at managing and reducing ulcers, some horse owners may not perceive this to be cost effective for preventative measures (Lybbert et al., 2007). Other management options should be taken into consideration, especially when considering the high prevalence of gastric ulcers in horses. Nutritional management in horses has recently been viewed to have a significant role on gastric ulcers (Reese et al., 2009). Other studies have shown that roughage source in combination with a concentrate diet can impact the prevalence of gastric ulcers. Lybbert (2007) compared alfalfa to Bermuda grass hay in twenty-four Quarter Horse yearlings of 12-14 months of age. Horses were divided into two groups, and were fed at 2.25% of their body weight, in a 50:50 ratio of the same concentrate with different forages. Horses were kept in small dry lots and exercised 3 days a week. After 28 days the horses were, allowed on pasture for a 21 day washout period, and then reassigned to the alternative treatment forage group. Horses fed alfalfa had reduced ulcer severity and compared to those fed coastal Bermuda hay (Lybbert et al., 2007).

A study by Nadeau (2000) showed similar results between alfalfa hay and bromegrass hay. Six mature cannulated 7-year old mix-breed mares were fed either an alfalfa-grain diet or bromegrass hay at 1.9% their body weight for a 14-day period. Horses were evaluated endoscopically before the trial, after being acclimated to their perspective ration, and upon completion of the trial to asses severity and number of ulcers present. The alfalfa based diet resulted in higher levels of volatile fatty acids, but

maintained the stomach at a higher pH (Nadeau et al., 2000). Data for pH was collected

via a pH electrode inserted in the stomach. In the alfalfa based diet, the mean pH of

gastric juice in the stomach ranged from 2.30 to 4.84 in comparison to the bromegrass

based diet where pH ranged from 1.95 to 5.12 throughout a 24-hour period. Horses on the

alfalfa diet also had less severe ulcers and a lower number of ulcers in the nonglandular

squamous region of the stomach (Nadeau et al., 2000).

   As a result of these studies, it is believed that the high calcium and protein content

usually present in alfalfa helps buffer the stomach and raise pH, protecting it from

developing gastric ulcers (Nadeau et al., 2000; Andrews et al., 2005). It is potentially

significant to understand the nutrient profile of a roughage source in order to help

improve management and prevention of gastric ulcers. More extensive research needs to

be conducted to understand the effects different forages and nutrient composition have on

reducing or preventing gastric ulcers in older horses and horses in more intensive

exercise regimens (Lybbert et al., 2007). Continued research in diet and management will

be important to future preventative and healing of gastric ulcers.

CHAPTER III

MATERIALS AND METHODS

Phase I – Gastric Ulcer Induction Phase

The protocol for this study was authorized by the Oklahoma State University Institutional

Animal Care and Use Committee (AG 14-23). In the first 7 days of the study, 21 horses

were managed under an intermittent feeding protocol to induce gastric ulceration

(Murray, 1994). This protocol was utilized in order to produce gastric ulceration in horses

for use in this trial (Table 1).

Management of horses

Twenty-one mature Quarter Horses (19 mares, 2 geldings) between the ages of 5 and 21

years old (mean age 13 years) and weighing between 429 and 648 kg (mean weight 523

kg) were used. Horses were sourced from two different locations, with the halter type (n

$= 10$) from a private facility and performance type ($n = 6$) from the Center of Veterinary

Health Sciences Vet Med Ranch. Horses were brought to the OSU facility 4 d prior to the

induction phase to allow ample time for acclimation to their environment and

management routine. Horses were individually housed in 12'x 12' stalls. During the 7 d

induction protocol, horses had *ad libitum* access to water with intermittent feeding of

prairie grass hay (Table 1). On d 0, horses were

fasted from 0700 to 1900 followed by an *ad libitum* fed period from 1900 to 0700 the

following morning for the 12 h periods of the induction protocol. On d 2 to 7, horses

were rotated at 0700 be either on the fed or fasted state to for 24 h periods. During fed

periods, horses were separated into 3 groups based on social behavior in prior

management and assigned to a dry lot with ad libitum access to water and hay. Each dry

lot had a round bale and trough to assure *ad libitum* access to hay and water. During the

fasting period, horses were returned to their respective stalls with *ad libitum* access to

water.

Table 1. Ulcer induction protocol feeding schedule[1]

	Protocol day						
	1	2	3	4	5	6	7
Hay available (h)	12	0	24	0	24	24	0
Hay withheld (h)	12	24	0	24	0	0	24

[1]Equine model of inducing ulceration in alimentary squamous epithelial mucosa (Murray, 1994)

Phase II - Feeding Phase

Gastric Ulcer Evaluation

On d 0, a gastroscopic examination was performed on 21 horses from Phase I.

Horses were fasted for a minimum of 12 hours to ensure an empty stomach for

gastroscopy. Water was provided *ad libitum* during the fasting period. Weight was

determined, by a floor scale, and recorded for each horse prior to gastroscopy. Horses

were sedated with xylazine (.25-.5 mg/kg BW) intravenously while being restrained in

stocks. The endoscope was placed and entered through the nasal meatus and continued to

the pharynx. Approximately 25 ml of water was passed through the scope to stimulate the

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horse to swallow, with the endoscope passing through the esophagus with swallowing. The stomach was insufflated with air after the scope entered the stomach, allowing for adequate visualization of the stomach lining. The stomach lining was evaluated for the presence and severity of gastric ulcers. Ulcers present were scored using a scoring system (Table 2) developed by MacAllister et al., (1997). After evaluation of the of the stomach lining, excess air was suctioned from the stomach and the scope was removed.

Experimental design

On day 0, following gastroscopic examination, 16 horses with an ulcer score of $\geq$ 1 in the greater curvature and $\geq$ 2 lesser curvature were selected. Horses were assigned to either a high fiber or low fiber group and fed their respective rations for 28 d to determine the effects of high fiber versus a low fiber concentrate on gastric ulceration. Evaluation of scores on d 0, 15, and 30 were scored later, blind and randomized. A scoring system (Table 2) developed by MacAllister et al., (1997) was used to assign gastric ulcer scores. Horses were paired by weight, gastric ulcer score, and type then randomly assigned to either the high fiber level group (HF) or low fiber level group (LF) in a randomized block design. Each group was fed on their respective ration for 30 days at 1% of BW in both concentrate and hay. Horses were fed twice a day at 06:00 and 18:00 to match common industry management. A*d libitum* access to water was also provided. Horses were kept in individual 12' x 12' stalls which were cleaned at a minimum of once per day to remove all feces and urine. All horses were turned out in groups in dry lots for a minimum of 2 h per day for free exercise.

Table 2. Gastric lesion scoring system[1]

Lesion severity score	Description
0	No lesion
1	Appears superficial (only mucosa missing)
2	Deeper structures involved (greater depth 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 haemorrhage or adherent blood clot

[1]A scoring system for gastric ulcers in the horse (MacAllister et al., 1997)

Diet

Commercially available feeds with differing fiber levels were selected for use in this trial. Rations were texturized feeds with a corn and oat base. Feed samples were collected and sent to Dairy One Forage testing laboratory (Ithaca, NY) for nutrient analysis (Table 3). The high fiber (HF) ration contained approximately 23% crude fiber (DM basis) and was formulated at an Oklahoma based feed mill, which provided funding for this project. The low fiber (LF) ration contained approximately 7% crude fiber (DM basis) and was formulated locally in Stillwater, Oklahoma. The HF ration had been reported to be effective for use in horses with ulcers. Horses which were clinically diagnosed with gastric ulcers were fed the HF ration for 30 days. Substantial improvement in gastric ulcer score was observed following gastroscopic evaluation. This data was not clinical or observed by Oklahoma State University. Based on this anecdotal

field data, the hypothesis was formed that feeding the HF ration as opposed to an alternative commercial ration with a substantially lower fiber level may help reduced the incidence and severity of gastric ulcers in horses.

Table 3. Nutrient analysis of LF (low fiber), HF (high fiber), and hay on a DM basis

	LF	HF	Prarie Grass Hay
DE[1], Mcal/lb	1.52 Mcal/lb	1.25 Mcal/lb	.90 Mcal/lb
Crude protein	17.7%	16.5%	5.1%
ADF[2]	10.3%	30.8%	42.0%
NDF[3]	18.2%	44.2%	67.2%
Lignin	2.1%	6.6%	4.0%
Crude fiber	6.9%	22.7%	28.3%
NFC[4]	55.3%	23.1%	18.3%
Starch	39.9%	8.8%	0.3%
Crude fat	3.2%	8.8%	2.2%
Ash	5.5%	7.4%	7.2%
TDN[5]	79.0%	70.0%	59.0%
Calcium	0.76%	1.03%	0.65%
Phosphorus	0.45%	0.41%	0.06%
Magnesium	0.20%	0.27%	0.22%
Potassium	1.11%	1.50%	0.69%
Sodium	0.22%	0.17%	

[1]DE = digestible energy
[2]ADF = acid detergent fiber
[3]NDF = neutral detergent fiber
[4]NFC = non-fiber carbohydrate
[5]TDN = total digestible nutrients

Gastric Variables Measured

Gastroscopic examination was performed on d 0, 15, and 30 of the trial to evaluate the occurrence of gastric ulceration. During this time, a stomach fluid sample was also extracted from the gastric antrum of the stomach during fasting and pH was measured and recorded on site with a portable pH meter (SevenGo™; Mettler, Toldedo, OH). Body weights were also recorded on d 0, 15, and 30 prior to gastroscopy.

Blood collection

Blood was collected via venipuncture on d 0, 15, and 30. Blood was collected prior to gastroscopic examination for red blood cell counts (RBC). Samples were immediately transported to the Oklahoma State University Center for Veterinary Health Sciences for evaluation of RBC. Blood was collected for cortisol evaluation on d 0, 15, and 30 approximately an hour before evening feeding and two hours after feeding. Samples were allowed ample time to clot and centrifuged at 1200 RPM for 10 min to allow separation of blood serum. Serum was stored in a freezer at -40º C until later analysis. Approximately 125 μL of serum were analyzed with a chemiluminescent enzyme immunoassay for cortisol in the IMMULITE® 1000 (Siemens Medical Solutions USA, Inc. Malvern, PA) to measure cortisol concentration found in blood serum.

Statistical analysis

Data for all dependent variables were analyzed using the General Linear Models procedure for repeated measures of SAS (SAS 9.4, 2012). When the F-test was significant (P, 0.05), the least significant difference (Steel and Torrie, 1960) was used to differentiate the difference between treatment means. The statistical model included the

effects of diet (HF or LF) on gastric ulcer score, pH, cortisol, RBC and their interactions. A covariate analysis was included to control the effects of other variables. Data is reported as means.

CHAPTER IV

RESULTS

Gastric ulcer score

Gastric ulcer scores were assigned by a licensed veterinarian with extensive experience evaluating gastric ulcers in horses. All scores from d 0, 15 and 30 were assigned blindly. Two scores were given per horse on each d. One score represented ulcers in the greater curvature (**GC**) of the stomach, with the other score representing the lesser curvature (**LC**) of the stomach. Of the 21 horses from the gastric induction phase (Phase I), 20 out of 21 horses had gastric ulcer scores in the GC and LC $\geq$ 1. Ulcers were only present in the nonglandular mucosa of the stomach, which is similar to the findings of other studies which utilized the intermittent feeding induction protocol (Murray and Grady, 2002; Murray and Eichorn, 1996; Murray, 1994). In Phase II no significant diet effects ($P > 0.05$) were observed between ulcer score and diet type in GC and LC (Figures 1 and 2). However, a decrease in ulcer scores was observed over time in the GC ($P < 0.05$) and LC ($P < 0.05$) between d 0 and 15 as well as between d 0 and 30 (Figures 1 and 2).
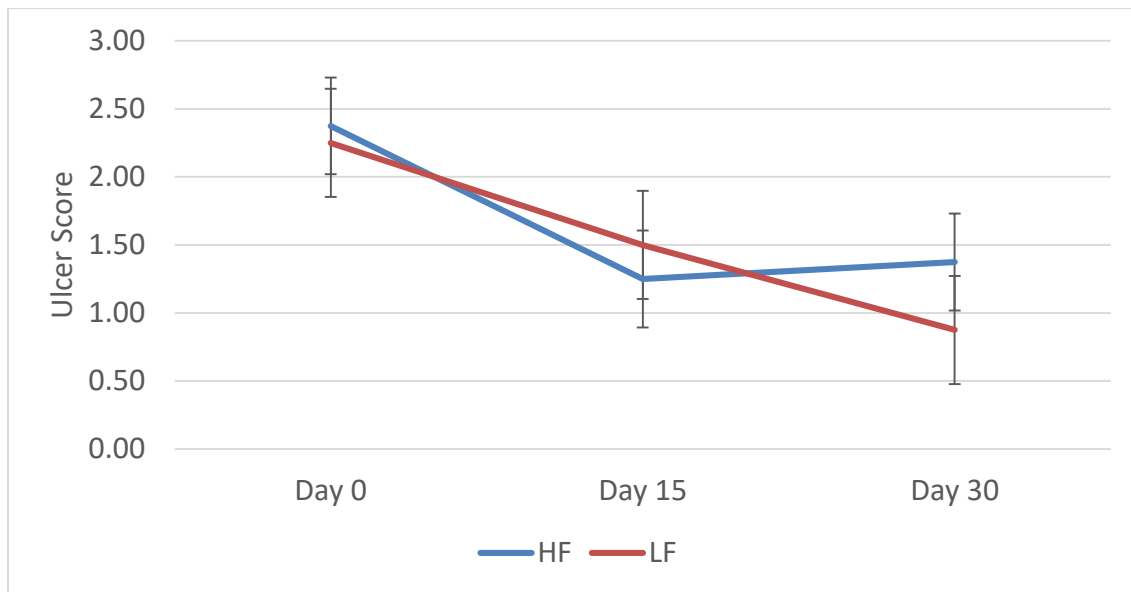
Figure 1. Greater curvature ulcer scores in horses fed the high fiber or low fiber diet at d 0, 15, and 30. Gastric ulcer scores in both diets decreased ($P < 0.05$) between d 0 and 15 as well as between d 0 and 30. Means within a day did not differ ($P > 0.05$) between the HF and LF.
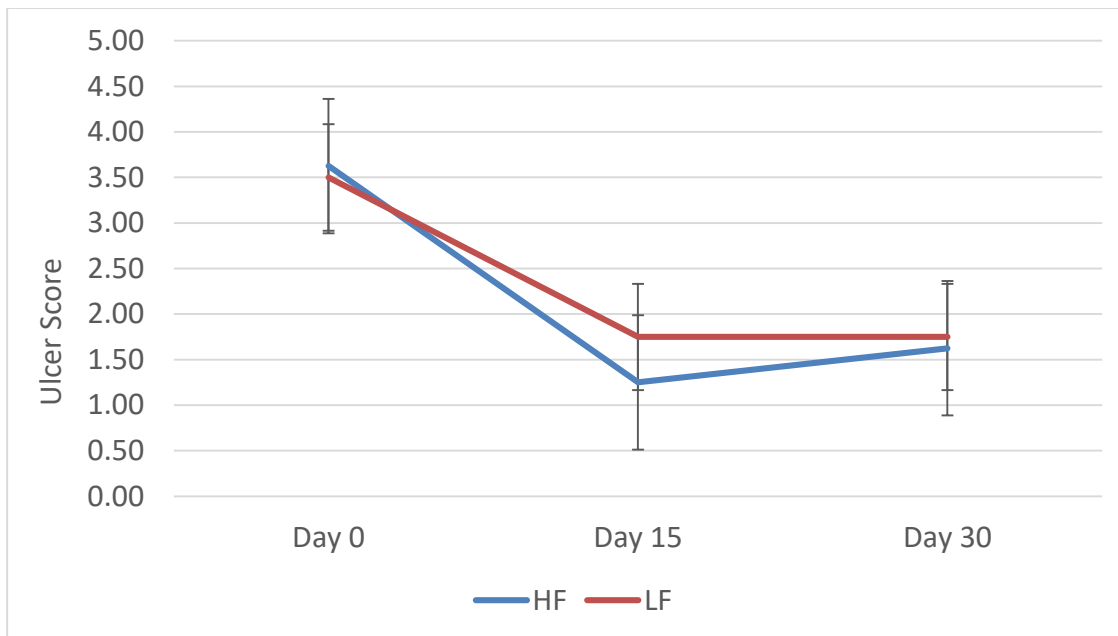

Figure 2. Lesser curvature ulcer scores in horses fed the high fiber or low fiber diet at d 0, 15, and 30. Gastric ulcer scores in both diets decreased ($P < 0.05$) between d 0 and 15 as well as between d 0 and 30. Means within a day did not differ ($P > 0.05$) between the HF and LF.

Stomach fluid pH

  Stomach fluid pH was collected on d 0, 15 and 30 from the antrum of the stomach. Horses fed LF had a higher ($P < 0.05$) mean pH on d 15 (Figure 3). Within pH, there was no significant interaction ($P > 0.05$) between diet and day of the trial. Differences were also seen between stomach pH and horse type, with the halter type showing a higher ($P < 0.05$) mean stomach pH (Figure 4). When interpreting this data, it is important to consider different group sizes of performance type ($n = 6$) and halter type ($n = 10$). Mean stomach pH for halter type horses was 5.10, 4.53, and 4.74 for d 0, 15, and 30, respectively. Mean stomach pH for performance type horses was 2.14, 3.06, 5.25 for d 0, 15, and 30, respectively.
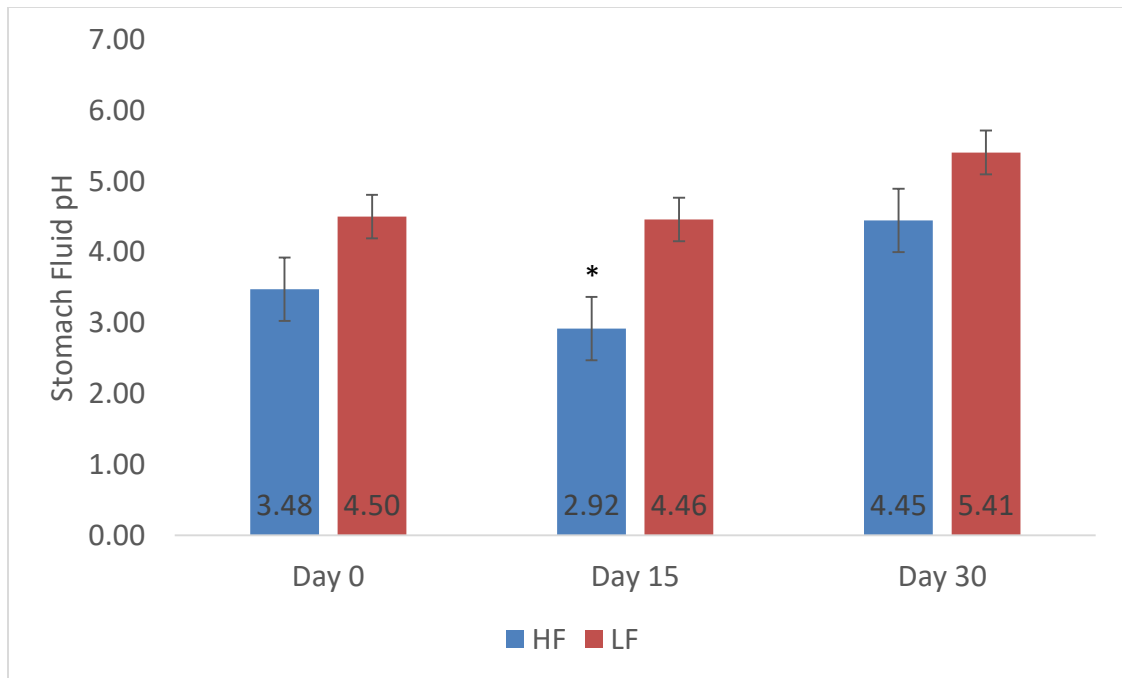
Figure 3. Stomach fluid pH in horses fed the high fiber or low fiber diet at d 0, 15, and 30. *Means differ ($P < 0.05$) between diets within a day.
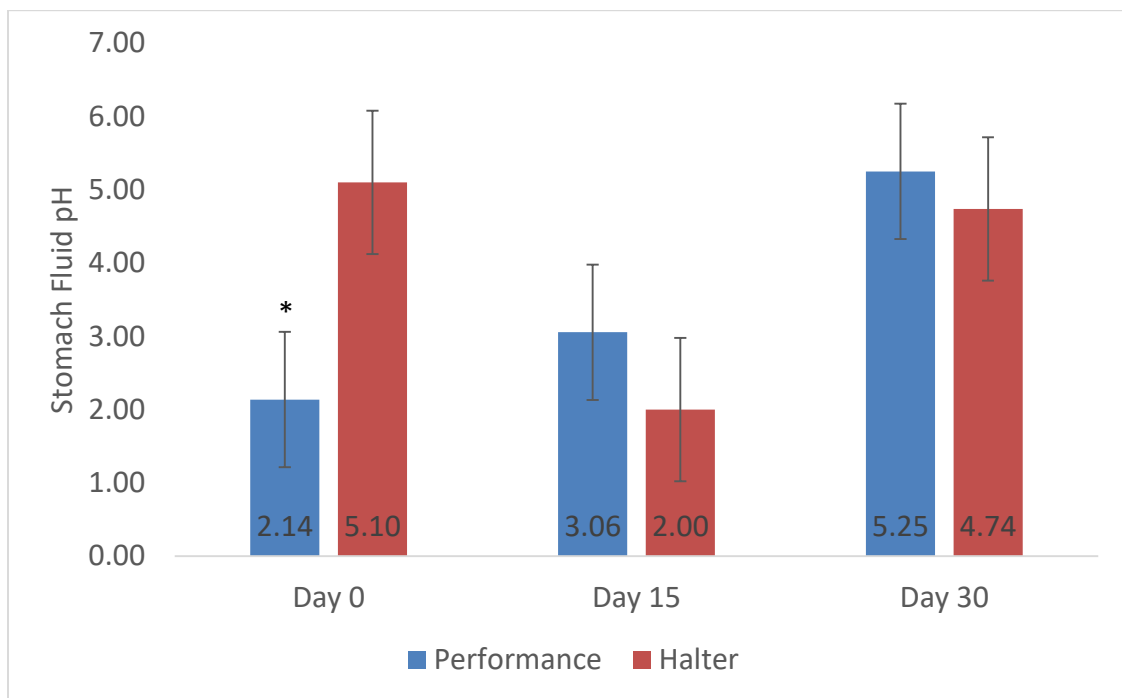

Figure 4. Stomach fluid pH in performance and halter horses fed the high fiber and low fiber diet at d 0, 15, and 30. *Means differ ($P < 0.05$) between performance and halter horses within a day.

Cortisol

Cortisol was measured pre- and post- feeding on d 0, 15 and 30. No difference (P > 0.05) in pre-feeding cortisol concentrations were observed between diets on d 0, 15, and 30. Horses fed the LF diet experienced a decrease (P < 0.001) in pre-feeding cortisol concentrations between d 0 and 15 as well as between d 0 and 30 (Figure 5). The post-feeding cortisol concentrations were similar between the diets at d 0 and 30, however the HF tended to be lower (P < 0.10) compared to the LF at d 15 (Figure 6).
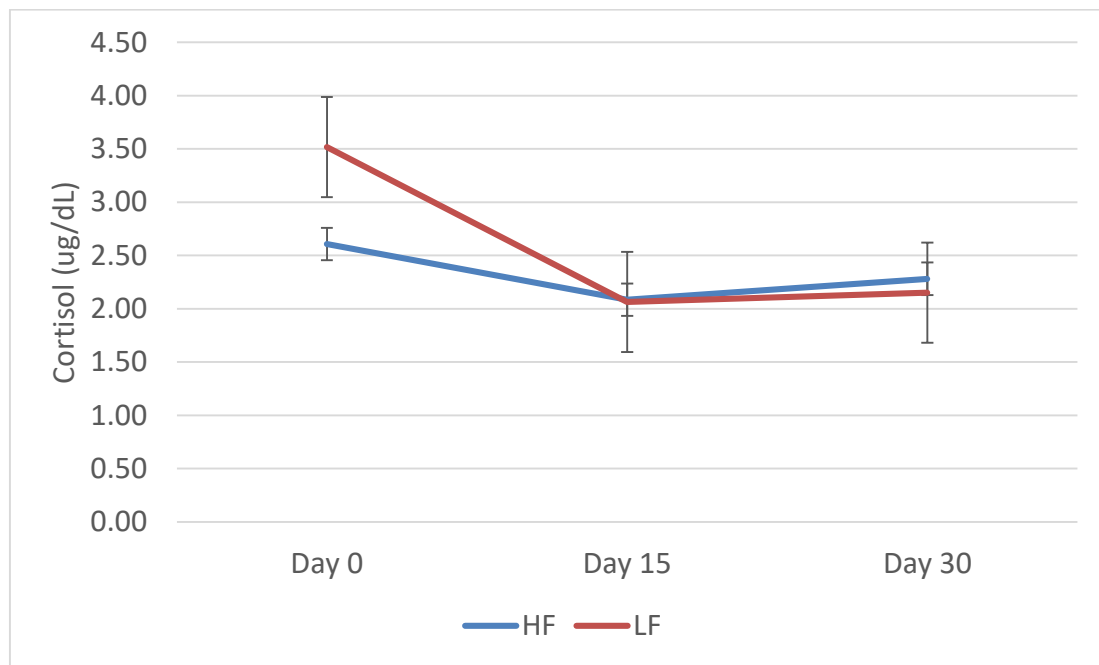

Figure 5. Cortisol concentrations pre-feeding in horses fed the high fiber or low fiber diet at d 0, 15, and 30. Cortisol concentrations in the LF decreased (P < 0.001) between d 0 and 15 as well as between d 0 and 30.
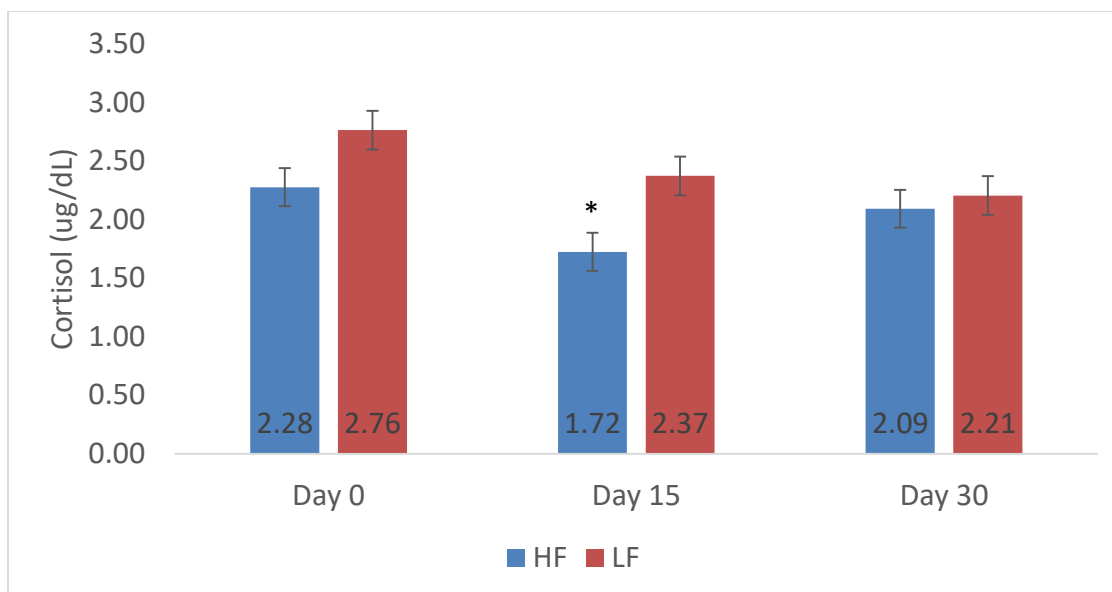
Figure 6. Cortisol concentrations post-feeding in horses fed the high fiber or low fiber diet at d 0, 15, and 30. *Cortisol concentrations in the HF group tended to be lower ($P < 0.10$) than the LF group on d 15.

Red Blood Cell Count (RBC)

Blood was collected on d 0, 15, and 30 for Complete Blood Count (**CBC**) analysis. All RBC levels reported were within a normal range (6.5-11.6 10^6/uL). No difference ($P > 0.05$) was observed in RBC level between diets at d 30. However, horses fed the LF diet had higher ($P < 0.05$) RBC levels at d 0 and 15 (Figure 7). Horses within the HF group had a decrease ($P < 0.05$) in CBC levels from d 0 to 15 (Figure 7).
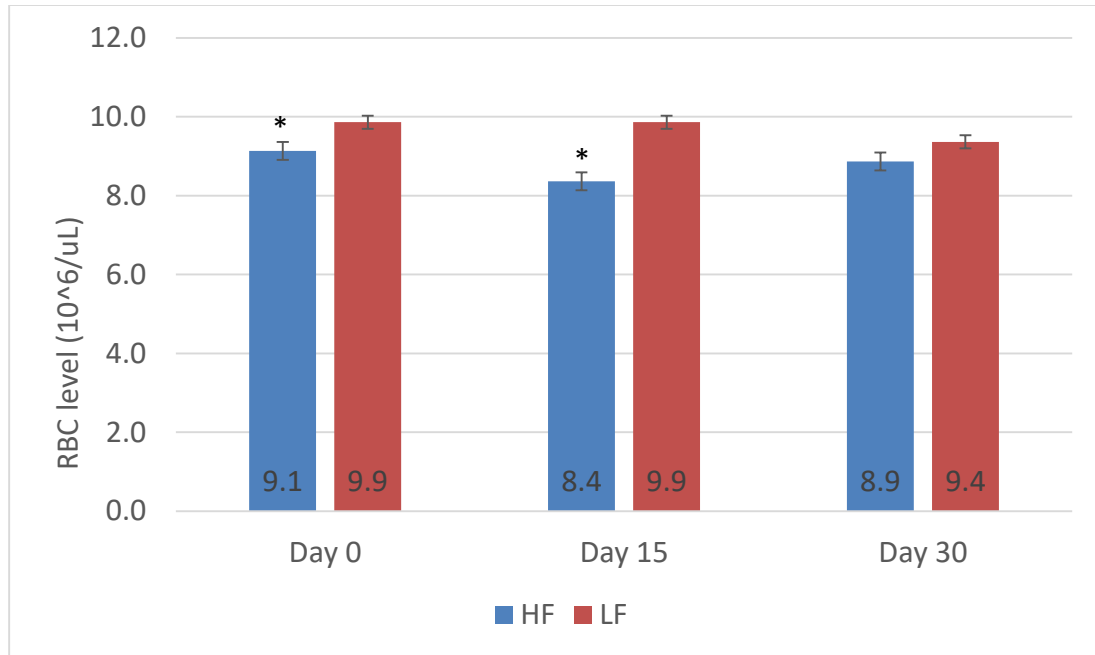
Figure 7. RBC levels in horses fed the high fiber or low fiber diet at d 0, 15, and 30.
*Means differ ($P < 0.05$) between diets within a day, RBC levels within the HF group
decreased ($P < 0.05$) between d 0 and 15.

CHAPTER V

DISCUSSION

Gastric ulcer score

The rate of healing (2-5 wk) seen in this trial is consistent with other research that utilized the same gastric ulcer induction protocol (Murray et al., 2001). All horses demonstrated improved ulcer scores throughout the trial, and no difference was found between diet groups. This suggests that fiber level in the concentrate portion of the diet did not have a significant impact on the rate of gastric healing found in the GC and LC. Regardless of the environment of the stomach, it has been observed that some ulcers have started healing even under the intermittent feeding protocol (Murray et al., 2001) and reducing acidity of the stomach was not always needed for healing (Murray et al., 1997; Murray et al., 2001). These findings suggest this protocol may not be suitable for evaluation of dietary treatments intended to alter gastric acid production.

As many have attributed the acidity of the stomach to the development of gastric ulcers, acid suppression therapy has been the predominant treatment in reducing the severity of gastric ulceration. This data is consistent with findings of Murray et al. (1997; 2001) who found this treatment may not be required to improve gastric ulcers. It is important to consider that this trial induced ulceration by intermittent feeding. It is possible that the pathophysiology of induced ulcers could be different than

those that are naturally occurring. Camacho-Luna et al. (2018) has reported that only

approximately 4% to 6% of nonglandular ulcers heal spontaneously, which is a much

lower rate of healing when compared to what was observed in this trial. Vatistas et al.

(1999) also reported that spontaneous healing is not commonly seen with horses in

vigorous training.

While acidity of the stomach does play a role in both induced and natural ulcers,

horses in this trial did not experience other environmental effects which can contribute to

the prevalence of gastric ulcers. For example, these horses were not maintained in

vigorous exercise routines, transported frequently, administered NSAIDs, or appeared to

be under chronic stress during the trial. Extensive information on horse's previous

management is unavailable aside from horses being managed on pasture. These

additional factors are commonly seen in the environments of performance and race

horses, where the highest prevalence rate (53-93%) of ulcers in the industry has been

observed. If horses were maintained in the same conditions seen in the performance

industry, the results of this trial could be more applicable to the industry. Unfortunately,

with the population size and availability, those factors were not included in this trial.

Stomach fluid pH

Stomach fluid pH values observed in this study are consistent with the range of

1.5-7.0 which are typically seen in horses (Andrews and Nadeau, 1999). Few gastric

ulcer studies have evaluated stomach fluid pH for comparison to this data. Horses in the

HF group had a lower stomach fluid pH values on d 15. Gastric fluid samples were

collected via endoscope from the gastric antrum, where pH is commonly low, after a

minimum of 12 h of fasting. While horses were restricted from feed during the fasting

period they were allowed *ad libitum* access to water. An explanation for differences seen in stomach fluid pH between the groups could be partly due to the amount of water consumed by each horse. While horses had *ad libitum* access, intake of water was not measured. The presence of water or saliva in the gastric fluid at the time of sampling could impact pH alkalinity. In addition, the process of scoping the entire group of horses was completed over the course of a day, which may leave horses sampled later in the day at a more acidic state compared to others scoped earlier in the day. Horses were scoped over the course of 5 hours.

The literature has reported various results regarding pH values. In a study evaluating 24 h pH levels in 5 horses, those restricted on feed did not show a difference in mean gastric pH values (Murray and Schusser, 1993). Murray and Schusser (1993) noted that no circadian differences in fasted gastric pH were seen in this study. However, they did not deny the possibility of a circadian rhythm in pH. However, others report a natural decrease in pH, specifically at 1:00-9:00 AM in horses on a feeding regimen (Reese et al., 2009). It is likely that a fluctuation seen in pH values in fed horses is affected by their feeding schedule. Horses in this trial were fed at 7:00 AM. However, they were fasted the morning of pH data collection.

The processing method and nutrient content of the HF and LF ration did not differ. While both rations were texturized, the additional portion of fiber, fat, minerals and vitamins in the ration was pelleted which may impact rate of passage. Additionally, horses may have spent less time chewing during concentrate intake, providing less saliva and consequently less buffering capacity to the stomach. It is commonly recognized that horses spend less time chewing while ingesting a concentrate when compared to a forage

(Bonin et al., 2007). A high fiber concentrate has a quicker passage rate when compared to a high starch concentrate (Métayer et al., 2004). A faster gastric emptying rate was also observed in a low starch concentrate compared to a high starch concentrate (Métayer et al., 2004). The diets used in this study, however, were not isocaloric. Others have observed no difference in gastric emptying when comparing high-fat and high-carbohydrate diets (Lorenzo-Figueras et al., 2005). Due to the results observed here, it is thought that gastric emptying rates will be similar as long as caloric content is comparable (Lorenzo-Figueras et al., 2005). The effect of fiber on rate of gastric emptying has been inconsistent in different studies depending on the species. For example, in pigs, a diet with a higher insoluble fiber content had a higher gastric emptying rate when compared to other isocaloric diets with lower fiber and higher starch content (Guerin et al., 2001). These varying results suggest that both nutrient content and caloric content of the diet could have an impact on gastric emptying.

A difference in stomach pH values when comparing horse type (performance vs halter) was also noted. Horse type was balanced between the HF and LF group, there were 5 halter 3 performance horses in each group. Halter horses are bred specifically for halter classes in stock type breed association shows, such as the American Quarter Horse Association. These horses are typically taller framed and heavier muscled when compared to other performance disciplines in the show industry. The halter group showed a higher mean pH, most noticeably on d 0. According to the literature, genetic differences in stomach pH have not been previously recorded in horses. These horses were all American Quarter Horses, but the genetic variation in bloodlines is very type specific within sectors of the western horse show industry. The difference seen in this trial may

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suggest that certain types of horses could be naturally more prone to a more acidic or basic stomach pH. However, with horse types being sourced from two different locations, previous management and environmental factors could also impact results. More research is needed in this area to support a suggestion of type differences on pH.

Cortisol

Cortisol was used to evaluate stress as links to a relationship with stress and gastric ulceration have been established (Malmkvist et al., 2012; Scheidegger et al., 2017). Hans Selye defined stress as any threat which would compromise homeostasis (Szabo et al., 2012). This threat can be either real or perceived from the external or internal environment. Stress can be difficult to interpret since a multitude of factors can influence an animal's ability to counteract a stressor. Factors such as age, gender, magnitude of the stressor, amount of exposure, and whether it is predictable or unpredictable can influence the degree in which a stress response is provoked (Gulati et al., 2015). A similar stressor can also be perceived differently from animal to animal.

The cascade of events which follow stress begins in the brain, and transfers to peripheral organs and body systems (Gulati et al., 2015). Release of cortisol has been identified as an adaptive response to a stressor, following a cascade of events that begins in the sympatho-adreno-medullary (**SAM**) system and hypothalamic-pituitary-adrenal (**HPA**) axis (Gulati et al., 2015). It has been established that the central nervous system is closely linked to the gastrointestinal tract through these various pathways (Konturek et al., 2011). In humans, multiple factors related to stress, have been identified that may lead to ulcers. Changes in gastric acid secretion, restricted mucosal blood flow, reduced

bicarbonate secretion, back flow of acid, lower healing of mucosa, and changes in gastric motility have all been identified as potential contributing factors (Konturek et al., 2011). These changes have been speculated to be an outcome of dysregulation of an array or neuropeptides that are involved in maintaining the integrity of the gastric mucosa (Konturek et al., 2011).

In this study, cortisol concentration fell within values observed in other studies with a range of 1.25-4.00 ug/dL reported (Ayala et al., 2012; Bohák et al., 2013; Casella et al., 2016). Cortisol concentration observed pre-feeding were lower in the HF group on d 0. No differences were noted in pre-feeding cortisol concentration between diet groups on d 15 and 30. When horses were paired and assigned in groups they were matched by type, weight, and gastric ulcer score. No previous knowledge of stress related factors were accounted for in the experimental design. Differences seen on d 0 could be attributed to the HF group's ability to counteract the stress of the induction phase. However, the lack of differences seen in pre-feeding cortisol concentration etween diet groups on d 15 and 30 suggests that the LF group was able to adapt their environment.

When evaluating cortisol concentration post-feeding, the HF group tended to be lower compared to the LF group on d 15. This difference may suggest that the HF diet had interaction with the neuropeptides involved in the stomach, which would communicate back to the HPA axis. Drawing a conclusion in this scenario is difficult to attribute to one component of this study, due to the complex nature of the stress system and its interaction with the gastric system. Also, it is important to recognize that circulating cortisol concentration can change quickly. Therefore, one sample taken at a specific time period could prove as a weakness for speculation. Measurement of more

parameters related to stress, in addition to more frequent sampling times, would be needed to make this a stronger conclusion.

RBC

Horses with gastric ulcers have been reported to be anemic (Hepburn, 2011). Due to this potential relationship, RBC was evaluated. All horses in this trial were noted to have RBC within a normal range of 6.5-11.6 10^6/uL (Antech Diagnostics, Oklahoma State Diagnostic Laboratory). However, the LF group had higher RBC at d 0 and 15. Additionally, within the HF group RBC decreased significantly from d 0 to 15. It is difficult to determine which variable in the trial could be impacting this level, however there is evidence of nutritional effects on RBC (Agina et al., 2017) which could contribute to the results seen here. Many other factors, though, have also been noted to attribute to hematological parameters such as exercise, training age, sex, breed, infection, temperature, and reproductive status (Agina et al., 2017). These findings are not physiologically significant, however, with horses reporting within normal range.

Conclusion

This study did not find a significant relationship between the level of fiber in the diet and severity of gastric ulcers. Our data observed that horses fed both a HF and LF diet improved in gastric ulcer score over time. Given the protocol used to induce ulcers, it was expected that both groups would improve in gastric ulcer score to some degree over the course of the study. We anticipated that the HF diet may improve gastric ulcers more rapidly. However, horses in this study had a faster rate of healing in gastric ulcer scores in this trial than what was predicted. To evaluate the effect of diet on gastric ulcer score,

performance horses (racing, showing, etc.) with naturally occurring ulcers should be used.

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