GASTRIC ULCER SYNDROME IN EXERCISING HORSES FED DIFFERENT TYPES OF HAY

A Thesis

by

TRAVIS CRAIG LYBBERT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2007

Major Subject: Animal Science

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ABSTRACT

Gastric Ulcer Syndrome in Exercising Horses Fed Different Types of Hay.

(May 2007)

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Equine gastric ulcer syndrome (EGUS) is highly prevalent in horses and most commonly found in racing and performance horses. This condition may negatively impact the health and athletic performance of affected horses (Vatistas et al. 1999). Proton pump inhibitors are commonly used to treat EGUS, however, a less expensive method, such as a change of diet, may give similar results. Alfalfa hay may offer some buffering capabilities within the stomach (Nadeau et al. 2000). The objective of this study was to further investigate any possible antiulcerogenic properties of alfalfa hay. Twenty-four Quarter Horse yearlings, 12-16 months of age, were utilized in this study. The 77-d experiment consisted of two 28-d periods separated by a 21-d wash-out period. Horses were endoscopically examined at the beginning and end of each period and blocked into two treatment groups. Treatment 1 included coastal bermuda grass (CB) hay and Treatment 2 included alfalfa hay as the only forage source. Horses were fed in stalls, housed in small dry lots, and subjected to an exercise regimen using a mechanical horse-exerciser.

A significant effect of diet, was observed on ulcer score ($P < 0.05$). CB hay–fed yearlings experienced an increase in ulcer score severity compared to that of alfalfa hay–fed yearlings. Significant healing did not occur during the wash-out period, but horses experienced a significant increase in ulcer score severity ($P < 0.05$). The outcome of this study suggests that alfalfa hay does have antiulcerogenic capability.

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CHAPTER I

INTRODUCTION

Equine gastric ulcer syndrome (EGUS) is highly prevalent in horses and most commonly found in racing and performance horses. This condition is recognized as a health problem in horses and can be detrimental to a horse's athletic performance. Potential negative impacts on the health and athletic performance of affected horses are documented (Vatistas et al. 1999). In fact, the leading complaint among horse owners and trainers is the declining performance of affected horses (Mitchell 2001). The increased availability of endoscopic equipment suitable for performing equine gastroscopy has facilitated more routine evaluation of horses for EGUS. Recent studies have reported prevalences as high as 93% among racehorses (Murray et al. 1996; Vatistas et al. 1999 'b'; Rabuffo et al. 2002) and over 60% of other performance horses have ulcers of varying severity (McClure et al. 1999). Murray and Eichorn (1996) also reported an increase from 93% to 100% prevalence in horses, which were consistently racing. Several factors associated with the development of EGUS include feed deprivation, stall confinement, increased intraluminal pressure with dorsal displacement of acid during exercise, intensive exercise, retention gastric acid resulting from functional or mechanical gastric outflow obstruction, and diet (Murray et al. 1996, Vatistas et al. 1999 'a', Lorenzo-Figuerus et al. 2002, and Merritt 2003). These factors may be directly linked to excessive acid secretion and decreased pH, which increases the opportunity for acid-induced injury.

This thesis follows the style of the Journal of Equine Veterinary Science.

Most gastric lesions develop in the squamous epithelial lining of the upper stomach, predominantly along the margo plicatus, where the only barriers against acid are intercellular tight junctions and saliva. The inadequate glandular mucosal protective mechanisms leave the stomach vulnerable (Murray 1999). The relationship between gastric ulcers and increased acidity is supported by the healing of gastric ulcer development following administration of proton pump inhibitors (Murray et al. 1997). Research has demonstrated that if provided a more basic environment (i.e., reducing gastric acidity by raising pH >4), stomach ulcers will heal spontaneously (Murray et al. 2001).

The relationship of diet and gastric ulcers has been the focus of numerous investigators. Nadeau et al. (2000) proposed that proteins in alfalfa may offer some buffering capabilities within the stomach. The results of the study demonstrated a strong correlation between horses fed alfalfa hay and lower degree of gastric ulceration.

The objective of this study was to further investigate any possible antiulcerogenic properties of alfalfa hay compared to coastal bermuda grass (CB) hay, in horses confined and subjected to forced exercise. It is hypothesized that an alfalfa hay-grain diet would lead to less severe gastric ulceration compared to a coastal hay-grain diet.

CHAPTER II

REVIEW OF LITERATURE

The functional anatomy of the equine stomach predisposes it to gastric ulceration. The equine stomach is divided into two distinct regions, the esophageal or non-glandular region and the glandular region. The esophageal region is covered by stratified squamous epithelium similar to the esophagus, and the glandular region contains glands that secrete hydrochloric acid, pepsin, bicarbonate, and mucus. These two regions are also known as the upper and lower stomachs respectively. The equine stomach continuously secretes variable amounts of hydrochloric acid throughout the day, with or without the presence of feed. Prolonged exposure to gastric acids leaves the stomach vulnerable to injury. Injury occurs to a lesser extent in the lower glandular portion of the stomach because of inherent mechanisms of protection. The glandular epithelium is protected by secretion of mucus and bicarbonate that forms a protective layer. Although ulcers may be found in both regions of the stomach, they occur more frequently in the non-glandular epithelium of the upper stomach which has more limited protection against acid (Murray et al. 1989; Vatistas et al. 1999 'b'). Gastric ulcers are frequently found along the margo plicatus because this area is constantly exposed to gastric acid. Several acids (HCl, VFAs, and bile acids) have been shown to cause damage to the nonglandular region. These acids cause inhibition of cellular sodium transport, cell swelling, and eventual ulceration while at a pH ≤ 4 (Nadeau et al. 2003, Berschneider et al. 1999). In contrast, glandular ulceration is typically not due to increased acidity, but rather the result of a compromised mucosal defense. The protective layer can be impaired by substances such as phenylbutazone (PBZ), a commonly used non-steroidal-anti-inflammatory drug in equine medicine (Monreal et al. 2003).

Human gastric ulceration is not analogous to ulceration of the equine squamous epithelium. Equine squamous ulcers more closely resemble gastro-esophageal reflux disease (GERD) in humans, because of the histological similarity to esophageal mucosa (Berschneider et al. 1999). Clinical signs associated with GERD have included chest pain (heartburn), irritability, refusal to eat and failure to thrive. Performance horses may experience similar symptoms particularly because most are not fed before exercise such that gastric acidity may be increased by the loss of buffering effects of feed. The esophageal sphincter of the horse is designed so as to prevent eructation of gases or ingesta, which does not allow relief in that fashion.

Availability of endoscopic equipment suitable for performing equine gastroscopy has facilitated the diagnosis and evaluation of horses for EGUS. Recent studies have documented prevalences as high as 93% among racehorses (Hammond et al. 1986; Murray et al. 1996 and 1989; Vatistas et al. 1999 ‘a’; Rabuffo et al. 2002; Jonsson and Egenvall 2006) and over 60% of other performance horses have ulcers of varying severity (McClure et al. 1999). Other methods for detecting ulcers are also being explored. O’Conor et al. (2004) recently evaluated a sucrose absorption test to potentially diagnose gastric ulcers. The test was found to be highly sensitive and specific in a small group of horses. A serum based sucrose permeability test has also been recently reported (Hewetson et al. 2006).

Many horses with ulcers show no outward clinical signs and their condition can only be detected via endoscopy. Clinical signs manifested by horses with EGUS include anorexia, weight loss, “sour” attitude, poor hair coat, and reduced level of performance (Murray 1992 and 1994**;** Vatistas et al. 1999 ‘a’). Sandin et al. (2000) reported an association between gastric ulceration and colic. Further research is needed to better understand the correlation between the presence of ulcers and its impact on the health and performance of horses. It is also unclear to what extent ulcer severity is related to the level of discomfort or reduced performance of horses.

A number of scoring systems have been reported to describe gastric ulceration in horses (Andrews et al. 2002; MacAllister et al.1997). An industry-sponsored council of experts proposed a system that is tabulated below (Table 1) (Merritt 2003).

Table 1. Ulcer scoring system proposed by The EGUS Council

Severity Scoring	
Grade 0	Epithelium is intact throughout; no hyperemia, no hyperkeratosis (yellowish color, sloughing)
Grade 1	Mucosa is intact but there are areas of hyperemia and/or hyperkeratosis
Grade 2	Small, single or multi-focal erosions or ulcers
Grade 3	Large, single or multi-focal ulcers, or extensive erosions and sloughing
Grade 4	Extensive ulcers, with areas of deep submucosal penetration

Acid injury has been implicated as a cause of EGUS, and several risk factors for its development have been identified. Diet has been isolated as a causal factor for EGUS. High starch diets contain large concentrations of digestible carbohydrates resulting in an increased HCl production because of elevated serum gastrin concentrations. Small amounts of VFA are also produced which causes damage to the nonglandular mucosa at a low pH (Nadeau et al**.** 2000 and 2003). McClure et al. (1999) found a strong association between ulceration and horses fed less hay and increased concentrate. Feed deprivation has also been shown to cause and to increase the severity of gastric ulcers (Murray 1994), (Murray et al. 1996 and 2001), presumably because a continuous flow of saliva and ingesta help to buffer the stomach. Erosions and ulcers were induced in equine gastric squamous epithelium through feed deprivation after prolonged exposure to gastric acid.

Vatistas et al. (1999 'b') found an association between the presence of gastric ulceration and decreased performance. The study consisted of 194 racehorses in training. The effect on performance appeared to be 'all or nothing', in that poor performance was associated with the presence of gastric ulcers, independent of their severity or number of ulcers. Similar results have been observed in humans suffering from GERD (Murray 1994). Horses in training are subject to all forms of ulcer inducing stressors such as intermittent feeding, confinement, high starch diets, and intense training. Evidence exists that stall confinement alone is sufficient to induce ulceration (Murray and Eichorn 1996**)**. Furthermore, transportation has also been shown to be a causal factor (McClure et al. 2005 'a'). Ten control horses were maintained on-site with no changes in management and ten horses were transported for 4 hours to a location where they were housed for 3 days. The horses were fed and exercised twice daily. The horses which were subjected to a simulated show or training environment experienced a significantly higher incidence of ulceration than the control horses. Evidence exists that horses running on a high-speed treadmill have increased abdominal pressure and decreased stomach volume (Lorenzo-Figuerus and Merritt 2002). The authors speculate that during exercise the stomach is compressed such that acid from the glandular mucosa is pushed dorsally into the nonglandular area. Therefore, exercise in horses could promote increased exposure of the stratified squamous mucosa to acidic contents, which ultimately leads to ulceration. Additionally, serum gastrin concentration has been shown to increase in exercising horses, which leads to increased HCl production (Furr et al. 1994). Jonsson and Egenvall (2006) found that horses in preparatory training and those that had raced during the last month had a significantly higher risk of having ulcers than did horses that were fit for racing but had not raced during the last month.

Diagnosis and treatment of EGUS has taken on greater importance since many horses have high economic value, particularly in the racing and performance industry. Numerous avenues of treatment have been proposed such as antacids, acid secretion suppressors, coating agents, and synthetic hormones. Acid suppression therapy is currently the most popular treatment. It has been proposed that ulcers do not need additional help to heal, but that the squamous epithelial lesions will spontaneously heal if the proper environment is created (Murray et al. 2001). The most effective pharmacologic agent has been proven to be omeprazole (Orsini et al. 2003; McClure et al. 2005 'b'). An oral paste of this drug inhibits gastric acid secretion for nearly 24 hours through binding reversibly to the H+/K+ pump in the parietal cells (Fellenius et al. 1981). The decreased secretion creates a less acidic stomach, which is more conducive to healing. Although proton pump inhibitors are advantageous, there are a number of concerns about the long-term use of these agents. First, HCl is essential for proper ingesta breakdown; therefore, research is needed to explore the possibility that acid secretion suppressors may interfere with total digestible energy absorption. Second, removing the protective antimicrobial effects of HCl may predispose to infectious disorders, such as clostridial diarrhea or respiratory infections (Dial 2005; Laheij 2003). Third, maintenance therapy with omeprazole is expensive and often cost-prohibitive for horse owners.

Proper management can eliminate several factors, which contribute to ulceration, but some aspects of a horse's life cannot be changed. Examples of this are turnout and time off from training. Murray and Eichorn (1996) found that horses turned out on pasture experience significant healing. After inducing ulcers in six horses through stall confinement and feed deprivation, a seven-day turnout period resulted in all but one horse experiencing dramatic healing. Grazing allows for a continuous flow of saliva and ingesta that help to buffer the stomach acid throughout the day. Allowing grazing may not be practical or feasible for some horse owners and trainers. Most performance horses must be consistently trained at intense levels in settings where turnout is not available. Because the circumstances of intensive training enhance the risk of gastric ulceration, many owners and trainers use omeprazole to treat and prevent gastric ulceration. Omeprazole has been proven to be effective for both treatment and prevention of EGUS (Murray et al. 1997, Vatistas et al. 1999 'b', Merritt 2003, Lester et al. 2005, McClure et al. 2005 (b)). Although effective, this cost of omeprazole renders it impractical for many horse owners to implement for treatment, prevention, or both; therefore, dietary solution would be practical and beneficial to the industry.

Alfalfa hay may have buffering capacity against HCl and VFAs (Nadeau et al. 2000). Six horses with gastric cannulae were fed a bromegrass hay diet or an alfalfa hay-grain diet following a crossover design. Stomach contents were collected immediately after feeding and hourly for the first 10 hrs and at hours 12 and 24. Horses fed alfalfa hay and concentrate experienced a reduction of number and severity of squamous mucosal ulceration relative to feeding a diet of bromegrass hay. The horses that were fed the alfalfa hay-grain diet were found to have an elevated VFA concentration; however, the pH of gastric fluid was significantly higher. The alfalfa-hay grain diet is high in fermentable carbohydrates, which causes an increased concentration of VFAs. At a low pH, VFAs cause acidification, uncoupling of sodium transport, cellular swelling, inflammation, and as an end result ulcers (Argenzio et al. 1991 and 1996; Nadeau et al. 2003). The authors speculated that the higher intragastric pH might have been attributable to the buffering effects of high concentrations of calcium and protein in the alfalfa hay fed to these horses. Despite the importance of these findings, systematic evaluation of the effects of feeding alfalfa hay to horses on the severity of gastric ulceration has not been reported. The purpose of the research reported here was to systematically investigate ulcer score severity in exercised yearling horses fed an alfalfa hay-grain diet vs. a coastal hay-grain diet.

CHAPTER III

EXPERIMENTAL PROCEDURE

Management of Animals

Twenty-four Quarter Horse yearlings, 12-16 months of age, owned by the Department of Animal Science at Texas A&M University were included in this study. Prior to initiation of the study, the horses were maintained on pasture and supplemented with a commercially available 15% crude protein pelleted feed (Producer's Cooperative Association, Bryan, TX). During that time, the yearlings were group fed twice daily and each received approximately 1.8 kg of concentrate/day and ad libitum grass. All yearlings were then gathered from the pasture and housed at the TAMU Horse Center, College Station, TX in accordance with the approved guidelines of the Institutional Animal Care and Use Committee. The horses were randomly housed in groups of four using 16m x 12m dry lots with identical footing, shelter, and water.

Experimental Design

This study was designed and conducted as a repeated measures crossover. The duration of the experiment was 77 days consisting of two 28-d periods separated by a 21-d wash-out period that was consistent with diet and environment for horses prior to the start of the study. At the beginning of period 1, the horses were examined endoscopically and blocked into two groups of 12 according to ulcer score. Each group was then assigned randomly to receive either Treatment 1 or Treatment 2. Treatment 1 included a coastal bermuda grass (CB) hay and Treatment 2 included an alfalfa hay, which will be discussed in more detail. Body weights of all horses were recorded at the beginning and end of each experimental period. Body weights were used to determine daily feeding amounts of individual horses. At the end of period 1, the horses were again examined endoscopically for gastric ulcers in order to evaluate changes in severity, and to assign an ulcer score. The endoscopist was blinded to the diet status of the horses. The horses were then returned to the original pasture in order to allow time for the stomach to return to its “normal state”. The third of four endoscopic examinations was performed prior to period 2, following the 21-d wash-out period; ulcer scores were again determined and body weights recorded. The yearlings were returned to the dry lots for another 28-d period during which those horses originally fed Treatment 1 in period 1 were switched to Treatment 2 and vice versa. A fourth endoscopic examination was performed at the end of period 2.

Endoscopic Examination

All horses were subjected to four endoscopic examinations. Feed was withheld for 18 hr prior to endoscopy and water was provided ad libitum. The dry lots were cleaned regularly during the fasting period to limit coprophagy, because intragastric feces can interfere with the endoscopic examination. A qualified veterinarian with established endoscopy skills performed these examinations. The yearlings were sedated intravenously with 1.5 – 2.0 ml of xylazine and a humane twitch was applied. The endoscope was passed via the nares to the stomach. Once within the stomach, the endoscope’s air channel was used to insufflate the stomach to maximize the surface area for efficient viewing. A minimum of four pictures were taken of the squamous epithelium and VHS recordings were taken by the endoscope. Malfunction of the videocassette recorder resulted in few VHS recordings being obtained. The squamous portion of the stomach was assigned an ulceration score of 0-4, based on the previously identified system with minor modifications scoring system. The scoring system did undergo some changes for this experiment prior to evaluation of results. Grade 2 was altered from “small, single or multi-focal ulcers, or extensive erosions or ulcers” to “small, single or multi-focal ulcers, or extensive erosions or ulcers, and no bleeding”. Grade 3 was altered from “large, single or multi-focal erosions or ulcers” to “large, single or multi-focal erosions or ulcer or bleeding”. These alterations allowed for a more critical system to assign and compare scores based on smaller, more precise indicators of EGUS.

Experimental Diets

The two treatments were as follows: Treatment 1 consisted of a coastal bermuda grass (CB) hay and a pelleted feed; Treatment 2 consisted of an alfalfa hay and the same pelleted feed as treatment 1. The concentrate was a commercially available feed used prior to this study (Table 2). The feed consisted of milo, wheat middlings, soybean, and appropriate minerals and vitamins. Although a digestion study was not conducted, this feed was calculated to contain approximately 2.75 mcal/kg of energy on a dry matter basis. The total diets consisted of 50% hay and 50% pelleted feed. Horses were fed at 2.25% of individual body weights daily. Horses were individually fed their assigned diets at 12-hour intervals (0600 hr and 1800 hr). Individual feeding stalls were utilized. The feeding stalls were 3m x 3m in size, and the cement floors allowed for complete recovery of any refusals. Free access to water was provided both in the dry lots and in the feeding stalls. The pelleted feed was offered first and any refusals were weighed and recorded. Hay was offered immediately following consumption of the concentrate feed and all refusals were also weighed and recorded. A maximum time period of 30 minutes was allowed for concentrate consumption and a maximum of 3.5 hours was allowed for hay consumption. At the conclusion of period 1 all horses were turned out on pasture for 21 days, using the same pasture and feeding program prior to the start of the study. Pasture grazing was available as before and the yearlings were group fed the concentrate twice daily as before. Each horse consumed approximately 1.8 kg of concentrate per day. One horse initially in the CB hay diet was eliminated from the study prior to being fed alfalfa hay for reasons other than intestinal disorders. Diets were switched and the identical feeding regimen was followed as in period 1. Concentrate and hay samples were taken daily throughout the study and analyzed at the conclusion of the experiment by period and by treatment (Table 3).

Table 2. Expected[a] nutrient concentration of the commercially available pelleted feed fed to exercising yearlings receiving different types of hay (As fed)

	Ingredients
Nutrient Name	As Fed
Protein %	13.00
Fat %	2.90
Fiber %	10.00
Ca %	0.70
P %	0.50
AV P %	0.19
ADF %	13.80
NDF %	30.70
Lys %	0.62
Met %	0.18
R.F. %	17.10
Salt %	0.70
K %	0.87
S %	0.17
Mg %	0.25
Mn ppm	99.44
Iron ppm	156.3
Cu ppm	32.47
Co ppm	0.79
Zn ppm	115.13
Iodine ppm	0.59
Se ppm	0.44
Vit A iu/lb	3018.99
Vit D iu/lb	210
Vit E iu/lb	38.1
Vit A added iu/lb	2080.45
Vit D added iu/lb	27.4
Vit E added iu/lb	210
RIBO mg/lb	1.57
NIAC mg/lb	27.94
PANT mg/lb	6.79
PYRD mg/lb	2.5
THIA mg/lb	5.77
FO A mg/lb	0.26
B 12 mcg/lb	3.2
BIOT mcg/lb	149.44
Zn:Cu ppm	3.55
DE H kcal/lb	1289.62
DIG Lys %	0.34
Na %	0.29
D.M. %	88.6
Valine %	0.42
Ca:P	1.4

[a]These values provided by feed manufacturer

Table 3. Analyzed nutrient profile of concentrate and hays fed to treatment groups (DM basis)

Nutrient	Concentrate	Alfalfa	CB
Dry Matter %	94.8	91.72	93.2
Protein %	15.81	20.63	14.48
Gross Energy (mcal/kg)	4.307	4.358	4.401
NDF %	35.55	40.08	68.63
ADF %	17.92	26.34	29.75
Calcium, %	0.88	1.38	0.43
Phosphorus, %	0.605	0.275	0.26
Magnesium, %	0.36	0.285	0.135
Potassium, %	1.085	2.395	1.94
Chloride %	0.54	0.64	0.74
Total Copper, ppm	48	8	9
Total Iron, ppm	31	306	93
Total Manganese, ppm	188	81	126
Total Sodium, ppm	4399	1824	1731
Sulfur, ppm	2388	3449	4765
Total Zinc, ppm	139	21	31

Feed Analyses

Feed and hay samples were analyzed for crude protein, gross energy, neutral detergent fiber, acid detergent fiber, and mineral content. Samples were dried in a forced air oven at 62 C° for 72 hours and ground in a Wiley mill with a 1 mm screen. All samples were analyzed in duplicate. Crude protein percentages were determined utilizing a [1]LEEKO Nitrogen Analyzer. The [2]Parr 6300 Calorimeter was used to determine gross energies. The percentages of neutral detergent fiber and acid detergent fiber were found utilizing the [3]ANKOM200 Fiber Analyzer. [4]Soil, Water and Forage Testing Laboratory of The Texas A&M University System conducted the mineral analyses. [5]Servi-Tech Laboratories of Amarillo, TX conducted the analysis of chloride.

[1,2,3] Animal Science Department of Texas A&M University, College Station, TX
[4] Soil, Water and Forage Testing Laboratory of Texas A&M University, College Station, TX
[5] Servi-Tech Laboratories, Amarillo, TX

Exercise

The horses were subjected to an exercise regimen using a mechanical horse-exerciser. The exerciser encouraged six horses to move simultaneously by using six rotating panels including inner and outer fences. The circumference of the exercising path was 63.85 m. All horses were exercised Monday, Wednesday, and Friday of each week in groups of six. Horses within exercising groups were randomly changed each day. This regimen required that each horse begin exercising at a rapid trot for five minutes; approximately 3.50 m/sec. Following the trot, they walked for five minutes; approximately 1.82 m/sec. At this point, during weeks one and two, the horses repeated another five minutes of rapid trotting. A 10-minute walking cool–down period ended each exercising session. During weeks three and four, after walking and trotting, the horses were loped (instead of trotting) for five minutes, approximately 4.71 m/sec, followed by a 10-minute cool down.

Statistical Analyses

Feed intake data and ulcer scores were analyzed to determine significance using S-PLUS statistical software (Version 7.0, Insightful, Inc., Seattle, WA). For comparisons of continuous or ordinal data between groups, the Wilcoxon rank-sum test was used. To account for repeated measures over time and a discrete, ordinal outcome (i.e., the outcome was not a continuous variable that could be assumed to be normally distributed), regression methods known as generalized estimating equations (GEE) were used, with a Poisson link to account for the discrete data. A significance level of $P < 0.05$ was used for all analyses.

CHAPTER IV

RESULTS

Hay Intake Data

Dietary hay intakes were compared using linear mixed-effects models to account for the analysis of longitudinal (repeated observations among horses), continuous data. There was no significant difference in hay intake/kg of body weight by diet, irrespective of period (P= 0.0779); although horses fed CB hay tended to eat less hay/kg. Horses consumed more hay/kg of body weight during period 2 than period 1, and there was thus a significant period effect (P< 0.05) (Table 4). The period effect had to be accounted for in analysis because CB hay–fed horses consumed less hay/kg than alfalfa hay–fed horses during period 2, resulting in a significant difference of hay intake/kg of body weight by diet after accounting for period (P< 0.05). There was also no significant interaction effect of diet and time period on the quantity of hay consumed (P> 0.05).

Table 4. Mean daily hay intake by period and treatment (DM basis)

	Period 1		Period 2	
	CB	Alfalfa	CB	Alfalfa
Mean Intake (kg)	3.42[a]	3.43[a]	3.71[b]	3.99[b]
Se	0.31	0.34	0.54	0.24
Mean Intake (g/kg BW)	10.19[a]	10.15[a]	10.51[a]	11.48[b]
Se	0.40	0.20	1.22	0.09

[a,b]Rows means not sharing common superscripts differ (P< 0.05)

Mean nutrient intakes from hay, concentrate, and total nutrient intakes are found in tables 5, 6, and 8 – 11.

Table 5. Period 1 mean daily nutrient intake from hay by treatment (DM basis)

Nutrient	CB (g)	(g/kg BW)	Alfalfa (g)	(g/kg BW)
Protein	494.59	1.476	707.61	2.092
NDF	2344.17	6.995	1374.74	4.065
ADF	1016.16	3.032	903.46	2.671
Calcium	14.69	0.044	47.33	0.140
Phosphorus	8.88	0.027	9.43	0.028
Magnesium	4.61	0.014	9.78	0.029
Potassium	66.26	0.198	82.15	0.243
Chloride	25.31	0.076	21.89	0.065
Copper	0.03078	0.00009	0.02744	0.00008
Iron	0.31806	0.00095	1.04958	0.00310
Manganese	0.43092	0.00129	0.27783	0.00082
Sodium	5.92002	0.01767	6.25632	0.01850
Sulfur	16.29630	0.04863	11.83007	0.03498
Zinc	0.10602	0.00032	0.07203	0.00021
Total	3.42 kg	10.19	3.43 kg	10.15

Table 6. Period 2 mean daily nutrient intake from hay by treatment (DM basis)

Nutrient	CB (g)	(g/kg BW)	Alfalfa (g)	(g/kg BW)
Protein	537.21	1.520	823.14	2.367
NDF	2546.17	7.205	1599.19	4.599
ADF	1103.73	3.123	1050.97	3.023
Calcium	15.95	0.045	55.06	0.158
Phosphorus	9.65	0.027	10.97	0.032
Magnesium	5.01	0.014	11.37	0.033
Potassium	71.97	0.204	95.56	0.275
Chloride	27.45	0.078	25.54	0.073
Copper	0.03339	0.00009	0.03192	0.00009
Iron	0.34503	0.00098	1.22094	0.00351
Manganese	0.46746	0.00132	0.32319	0.00093
Sodium	6.42201	0.01817	7.27776	0.02093
Sulfur	17.67815	0.05002	13.76151	0.03958
Zinc	0.11501	0.00032	0.08379	0.00023
Total	3.71 kg	10.51	3.99 kg	11.48

Concentrate Intake Data

There was no significant difference in concentrate intake per kg of body weight by diet, irrespective of period (P> 0.05). There was a significant effect of period with respect to concentrate intake/kg (P< 0.05); horses consumed 1.18 g/kg more during period 2 than period 1. Even though a period effect was observed, there was no significant difference between the diet groups in the amount of concentrate consumed/kg of body weight, after accounting for period (P> 0.05).

Table 7. Mean daily concentrate intake by period and treatment (DM basis)

	Period 1		Period 2	
	CB	Alfalfa	CB	Alfalfa
Mean Intake (kg)	3.56^{a}	3.59^{a}	4.17^{b}	3.99^{b}
Se	0.25	0.37	0.37	0.23
Mean Intake (g/kg BW)	10.64^{a}	10.61^{a}	11.79^{b}	11.83^{b}
Se	0.05	0.08	0.14	0.07

^{a,b}Rows means not sharing common superscripts differ (P< 0.05)

Table 8. Period 1 mean daily nutrient intake from concentrate by treatment (DM basis)

Nutrient	CB Diet (g)	(g/kg BW)	Alfalfa Diet (g)	(g/kg BW)
Protein	563.51	1.682	567.37	1.678
NDF	1267.10	3.781	1275.77	3.772
ADF	638.72	1.906	643.09	1.901
Calcium	31.37	0.094	31.58	0.093
Phosphorus	21.56	0.064	21.71	0.064
Magnesium	12.83	0.038	12.92	0.038
Potassium	38.67	0.115	38.94	0.115
Chloride	26.38	0.079	22.97	0.068
Copper	0.17109	0.00051	0.17226	0.00051
Iron	0.11049	0.00033	0.11125	0.00033
Manganese	0.67009	0.00200	0.67467	0.00199
Sodium	15.67928	0.04679	15.78650	0.04668
Sulfur	16.98381	0.05068	12.37727	0.03660
Zinc	0.49544	0.00148	0.49882	0.00147
Total	3.56 kg	10.64	3.59 kg	10.61

Table 9. Period 2 mean daily nutrient intake from concentrate by treatment (DM basis)

Nutrient	CB Diet (g)	(g/kg BW)	Alfalfa Diet (g)	(g/kg BW)
Protein	658.58	1.864	650.19	1.870
NDF	1480.87	4.190	1462.00	4.205
ADF	746.48	2.112	736.96	2.120
Calcium	36.66	0.104	36.19	0.104
Phosphorus	25.20	0.071	24.88	0.072
Magnesium	15.00	0.042	14.81	0.043
Potassium	45.20	0.128	44.62	0.128
Chloride	30.83	0.087	26.32	0.076
Copper	0.19995	0.00057	0.19740	0.00057
Iron	0.12913	0.00037	0.12749	0.00037
Manganese	0.78313	0.00222	0.77315	0.00222
Sodium	18.32447	0.05185	18.09095	0.05203
Zinc	19.84908	0.05617	14.18407	0.04079
Total	4.17 kg	11.79	3.99 kg	11.83

Table 10. Period 1 mean daily nutrient intake from hay and concentrate by treatment (DM basis)

Nutrient	CB Diet (g)	(g/kg BW)	Alfalfa Diet (g)	(g/kg BW)
Protein	1058.10	3.158	1274.98	3.770
NDF	3611.27	10.777	2650.51	7.837
ADF	1654.88	4.938	1546.55	4.573
Calcium	46.05	0.137	78.91	0.233
Phosphorus	30.44	0.091	31.14	0.092
Magnesium	17.44	0.052	22.69	0.067
Potassium	104.94	0.313	121.09	0.358
Chloride	51.68	0.154	44.86	0.133
Copper	0.20187	0.00060	0.19970	0.00059
Iron	0.42855	0.00128	1.16083	0.00343
Manganese	1.10101	0.00329	0.95250	0.00282
Sodium	21.59930	0.06446	22.04282	0.06518
Sulfur	33.28011	0.09931	24.20734	0.07158
Zinc	0.60146	0.00179	0.57085	0.00169
Total	6.98 kg	20.83	7.02 kg	20.76

Table 11. Period 2 mean daily nutrient intake from hay and concentrate by treatment (DM basis)

Nutrient	CBI Diet (g)	(g/kg BW)	Alfalfa Diet (g)	(g/kg BW)
Protein	1195.79	3.384	1473.33	4.237
NDF	4027.04	11.395	3061.19	8.804
ADF	1850.20	5.235	1787.93	5.142
Calcium	52.61	0.149	91.25	0.262
Phosphorus	34.85	0.099	35.85	0.103
Magnesium	20.00	0.057	26.18	0.075
Potassium	117.17	0.332	140.18	0.403
Chloride	58.28	0.165	51.86	0.149
Copper	0.233339	0.000660	0.229321	0.000660
Iron	0.474164	0.001342	1.348428	0.003878
Manganese	1.250593	0.003539	1.096343	0.003153
Sodium	24.746483	0.070024	25.368715	0.072962
Sulfur	37.527232	0.106189	27.945575	0.080373
Zinc	0.694028	0.001958	0.655430	0.001877
Total	7.88 kg	21.94	7.98 kg	23.31

Table 12 contains the nutrient requirements of yearling horses in training. Mean daily nutrients provided to the horses in this study are found in tables 13 and 14. Nutrient requirements of the yearling horses were met > 110 %.

Table 12. Daily nutrient requirements of yearling horses in training

DE (Mcal)	CP (%)	Lys (%)	Ca (%)	P (%)	Mg (%)	K (%)	Vit A
22.7	13.6	0.57	0.48	0.27	0.09	0.28	0.23

Table 13. Period 1 mean daily nutrients provided to yearling horses in training by treatment

	CB Diet			Alfalfa Diet		
Nutrients	Requirements (g)	Provided (g)	%	Requirements (g)	Provided (g)	%
CP	1025.40	1141.90	111.4	1034.90	1386.60	134.0
Ca	36.19	49.40	136.5	36.53	86.00	235.4
P	20.36	32.60	160.1	20.55	33.50	163.0
Mg	6.79	18.70	275.4	6.85	24.50	357.7
K	21.10	114.20	541.2	21.30	132.40	621.6

Table 14. Period 2 mean daily nutrients provided to yearling horses in training by treatment

	CB Diet			Alfalfa Diet		
Nutrients	Requirements (g)	Provided (g)	%	Requirements (g)	Provided (g)	%
CP	1081.40	1204.27	111.4	1063.96	1425.38	133.9
Ca	38.17	52.10	136.5	37.55	88.38	235.3
P	21.47	34.44	160.4	21.12	34.43	163.1
Mg	7.16	19.68	274.9	7.04	25.25	358.6
K	22.27	120.24	539.9	21.91	136.12	621.3

Ulcer Score Data

The aim of this study was to evaluate and compare dietary influence on ulcer severity scores among yearling horses fed two different types of hay. There was no significant difference in ulcer severity score between the groups at day 0, irrespective of period ($P > 0.05$) (Table 10). There was no evidence that there was a significant difference of baseline scores (Period 2 – Period 1) by diet ($P > 0.05$). Ulcer scores at day

0 tended to be higher for period 2 (median, 2; range, 0 to 3) than for period 1 (median, 0; range, 0 to 3) but this difference was not significant (P> 0.05). Accounting for the paired structure of the data, the median difference of the baseline score at day 0 for Period 2 minus that for Period 1 was 0 (range, -2 to 3), and this difference was not significant (P> 0.05). This approach, however, still does not account for the repeated observations among horses, and so generalized estimating equations (GEE) were used for analysis. Using GEE, there was no significant difference between periods on the baseline score (P> 0.05). There was also no evidence of a significant difference between diets on baseline score (P> 0.05).

Table 15. Mean ulcer severity scores of horses by period and treatment

	Period 1		Period 2	
	Day 0	Day 28	Day 0	Day 28
Coastal Diet	0.8	1.1[a]	1.3	2.0[a]
Se	1.27	1.46	1.15	1.21
Alfalfa Diet	0.8	0.1[b]	1.5	0.3[b]
Se	1.27	1.23	1.31	1.29

[a,b]Values in same column with different superscripts are different (P< 0.05)

There was a significant effect of diet on ulcer score (P< 0.05) (Table 7). Horses fed CB hay had significantly higher ulcer scores. The effect of diet was strong, with an estimated effect of increasing the ulcer score by a score of 1.5 (95% confidence interval, 1.2-1.8). Accounting for period and repeated measures, the ulcer severity scores were significantly (P< 0.001) lower for horses in the alfalfa hay group than horses fed coastal bermuda grass hay. There was no significant effect of period (P> 0.05), therefore, controlling for its effects yielded the same result ($P < 0.05$). There was no significant interaction of diet and period ($P > 0.05$); thus, the effect of diet was the same regardless of period. In period 1, both treatment groups had mean ulcer scores of 0.8 on day zero. Those horses fed CB hay increased by 0.3 while horses fed alfalfa hay decreased by 0.7 at day 28. A similar pattern was observed in period 2; horses fed CB hay increased in ulcer score severity by 0.7 and alfalfa fed horses decreased by 1.2. When horses were fed CB hay, the overall change in ulcer score of both periods was + 0.5 over 28 days. However, when horses were fed alfalfa, the overall change in ulcer score was – 0.95. This comparison can be made since a period effect was not observed.

Among horses fed alfalfa, 12 had no ulcers at baseline and 11 had ulcer scores of 2 (N=6) or 3 (N=5). Of the 11 horses with ulcer scores > 0, all improved by at least 2 ulcer grades while on the alfalfa diet; 1 of 12 horses without ulceration developed gastric ulceration during the time it was fed alfalfa. In contrast, of the 12 horses fed CB hay that had ulcer scores > 0, 5 horses scores were improved and only 2 were improved by at least 2 grades; of the 12 horses with initial ulcer scores of 0 fed CB hay, only 3 remained free of ulcers and 7 developed ulcer scores ≥ 2.

Figure 1 shows gastric ulcer severity score for all 23 horses from both trial periods. At day 0: 12 horses were at an ulcer severity score of 0, 0 horses with a score of 1, 6 horses with a score of 2, and 5 horses with a score of 3. At day 28: 21 horses were at an ulcer severity score of 0, 1 horse with a score of 1, 0 horses with a score of 2, and 1 horse with a score of 3.

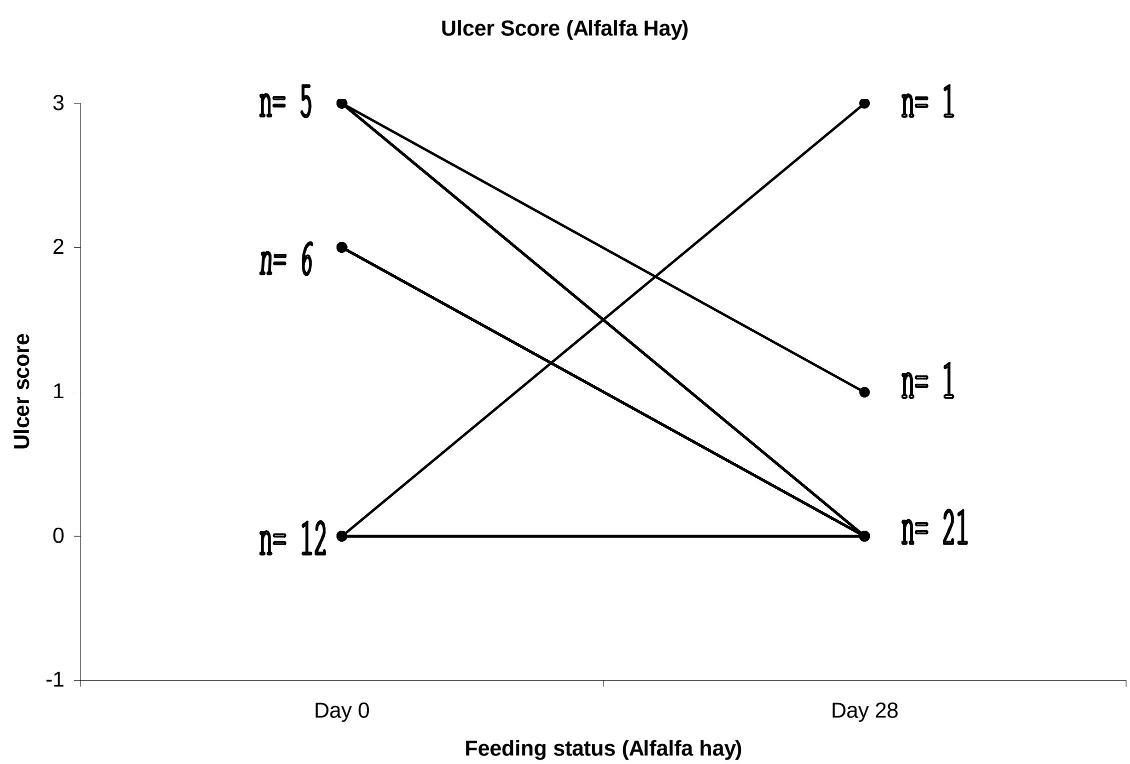

Figure 1 – Gastric ulcer severity score of 23 horses fed alfalfa hay.

Figure 2 shows gastric ulcer severity score for all 24 horses from the both trial periods. At day 0: 12 horses were at an ulcer severity score of 0, 2 horses with a score of 1, 6 horses with a score of 2, and 4 horses with a score of 3. At day 28: 4 horses were at an ulcer severity score of 0, 5 horses with a score of 1, 13 horses with a score of 2, and 2 horses with a score of 3.

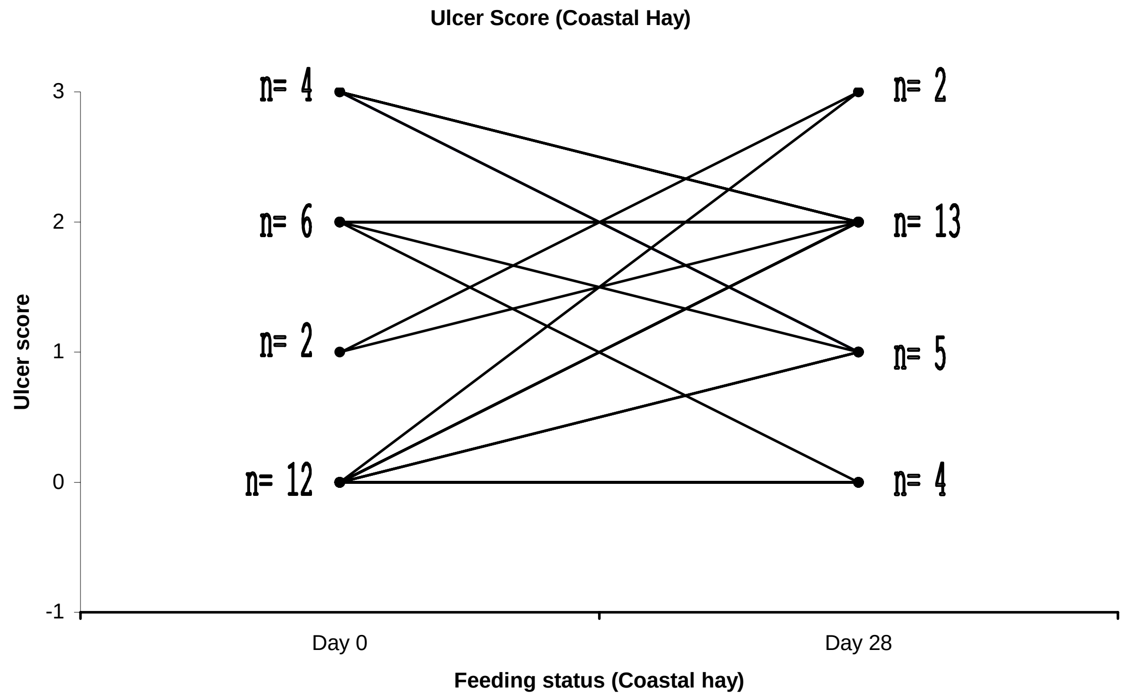

Figure 2 – Gastric ulcer severity score of 24 horses fed coastal hay.

Ulcer score severity of both treatment groups increased during the wash-out period. Horses previously fed alfalfa hay during period 1 experienced a significant change from 0.1 to 1.3 during the wash-out ($P< 0.05$). Horses previously fed CB hay during period 1 did not experience a significant change during the wash-out ($P> 0.05$), as average scores were evaluated to be 1.1 and 1.5. Post wash-out ulcer severity scores were not significant by treatment ($P> 0.05$).

Table 16. Wash-out period ulcer severity score

	Pre-washout	Post-washout	Change
Coastal Diet	1.1[a]	1.5[a]	0.4
Se	1.46	1.31	
Alfalfa Diet	0.1[b,c]	1.3[a,d]	1.2
Se	1.23	1.15	

[a,b]Values in same row with different superscripts are significant ($P < 0.05$)
[c,d]Values in same column with different superscripts are significant ($P < 0.05$)

There was a significant effect of diet on ulcer score, after adjusting for both the mean amount of hay consumed per kg of body weight and period ($P < 0.05$): the significance and the magnitude of the effect of alfalfa remained similar following adjustment, increasing the ulcer severity score by 1.5 (95% confidence interval; range, 1.1 to 1.9). There was again no significant effect of period ($P > 0.05$).

Tables 17 and 18 demonstrate the strong effects that diet had on gastric ulceration in the yearling horses. Overall only one horse from the alfalfa group increased in ulcer severity compared to ten horses from the CB group.

Table 17. Period 1 changes of ulcer scores

worse on alfalfa 0	same or better on alfalfa 12
worse on CB 5	same or better on CB 7

Table 18. Period 2 changes of ulcer scores

worse on alfalfa 1	same or better on alfalfa 11
worse on CB 5	same or better on CB 7

CHAPTER V

DISCUSSION

The outcome of interest was whether diet influenced change in ulcer score. Analyses of these data were complicated by repeated measures on individual horses and use of score data. Ulcer score data have outcomes that are ordinal, discrete data and thus could not be analyzed appropriately with repeated measures ANOVA. This study consisted of two periods, which meant that each horse had two (three observed changes if it is acceptable to compare changes before and after the wash-out) observed changes (two pairs of observations for a total of four observations per horse); therefore, the observations of change in ulcer score were not independent. This correlation among observations was accounted for by using GEE methods. The second complication was that the ulcer score data were not truly continuous data. To account for this correlation among observations, a Poisson link function was used in the GEE models.

Horses fed CB hay consumed less hay/kg of body weight than alfalfa in period 2 ($P< 0.05$). This may be explained by two different theories. Horses increased in body weight during the wash-out period. This weight increase may have been attributed more to fat gain than bone mass. Since horses were fed according to a percent of body weight, a high fat:bone ratio could result in an increased amount of feed provided than is actually required. The extra feed may have amounted to more than certain individuals could consume. Also, CB hay could have possibly been less palatable due to higher fiber content (68.6%). The combination of increased hay and lower palatability could have caused the increased refusals by the horses fed CB hay. Minimal refusals were recorded from horses fed alfalfa hay possibly due to the high palatability cause of lower fiber content (40.1%). The lower consumption of CB hay may also have been due to ulceration. The increased ulcer score severity of the horses fed CB hay may have led to a certain amount of anorexia. Concentrate intake/kg of body weight increased in period 2, for unknown reasons.

Degree and incidence of ulceration were influenced by diet ($P < 0.05$). CB hay–fed yearlings experienced an increase in ulcer severity compared to that of alfalfa hay–fed yearlings. It was hypothesized that alfalfa would prove to exhibit significant effects on horses but the results were even more remarkable than expected. Horses fed alfalfa hay during both periods1 and 2 dramatically improved in ulcer score severity. Similar results were reported by Nadeau et al. (2000) with horses fed an alfalfa hay-grain diet had less severe gastric ulceration than did horses fed a bromegrass hay diet.

Research has shown that equine gastric ulcers may heal with pasture turnout (Murray and Eichorn 1996). The three-week wash-out period in this study did not result in dramatic healing (Table 13). In fact, recorded scores were higher following the 21-day wash-out period of both treatments, with the magnitude of the increase greater for those horses that entered pasture following the alfalfa treatment than those following the coastal grass treatment (1.2 vs. 0.4). During the wash-out period, the horses fed alfalfa hay during period 1 experienced a significant change from 0.1 to 1.3 ($P < 0.05$) and the horses fed CB hay during period 1 did not experience a significant change from 1.1 to 1.5 ($P > 0.05$). The change of ulcer score during the wash-out period is surprising since the horses were returned to their natural environment and ample green grass was present, due to weather conditions and time of year.

The alfalfa hay contained 1.5 times the amount of protein and 3.4 times the amount of calcium than the CB grass hay (Table 3). These agents may have some buffering capabilities (Nadeau et al. 2000).

Table 19 contains the dietary cation anion differences (DCAD) of the forages. The alfalfa hay contained a larger cation concentration than the CB grass hay and a lower anion concentration. The CB hay contained a DCAD balance of 285.7 and the alfalfa hay contained a balance of 431.3. The comparative higher DCAD balance of the alfalfa hay diet may have caused a slight increase of gastric pH; which could potentially buffer the HCl produced in the stomach, lessening incidence of ulcers. Dairy cattle are routinely fed diets with positive DCAD balances to raise pH, or negative DCAD balance to lower pH (Riond 2001).

Table 19. Dietary cation anion differences (DCAD) of forages

Nutrient	Conversion Factor (CF)	CB %	mEq/kg	Alfalfa %	mEq/kg
Sodium	435	0.001731	0.753	0.001824	0.793
Potassium	256	1.94	496.640	2.395	613.120
Chloride	282	0.74	208.680	0.64	180.480
Sulfur	624	0.004765	2.973	0.003449	2.152
			CB hay		Alfalfa hay
DCAD (mEq/kg)			285.7		431.3

(mEq/kg of each mineral= % * CF), (DCAD= mEq/kg of (Na + K) - (Cl + S))

Whether or not the differences observed in ulcer score were due to protein intake, protein quality intake, calcium intake or DCAD balance, could not be determined in this study.

CHAPTER VI

SUMMARY AND CONCLUSION

EGUS in horses is most likely not a new condition. Many years ago, work horses ate grain meals with various roughage sources, and probably suffered a similar malady. Only in more recent years has the technology improved to allow evaluation of a condition that may have affected horses since they were domesticated, trained and used for a variety of purposes. The purpose of this study was to utilize the current technology and investigate possible antiulcerogenic effects of alfalfa hay.

Dietary influences on ulcer score severity were evaluated and compared in this study. In conclusion, horses fed CB hay had significantly higher changes in ulcer score severity (day 28 – day 0), even after adjusting for period and mean hay consumed per kg body weight. On average, horses fed CB hay increased in ulcer score severity and horses fed alfalfa hay decreased. The significance and the magnitude of the effect of alfalfa remained similar throughout both trial periods. Of the 24 horses fed alfalfa hay only one increased in ulcer score severity, vs. 10 of 24 horses fed CB hay. Contrary to previous research, the horses in this study did not experience healing during the wash-out period, in fact, ulcer score severity increased. With respect to diet, there were differences in mean amounts of hay consumed/kg of body weight and this varied by diet and period. During period 1, the amount of hay consumed on a kg of body weight basis did not differ, but in period 2 the horses at less CB hay/kg of body weight. Horses consumed more concentrate/kg of body weight during period 2 than 1, but this amount didn’t differ by diet.

A previous study indicated that feeding alfalfa hay reduced the severity of gastric ulceration in mature, resting horses with gastric cannulae (Nadeau et al. 2000). Unlike the study by Nadeau (2000), horses in both groups were fed concentrates, thereby indicating that the dietary effect was likely attributable to alfalfa (or its interaction with concentrate). Finally, the horses in this study were in light training, indicating that alfalfa can be effective for ameliorating or preventing gastric ulceration in young, exercising horses. The extent to which theses results apply to older horses or horses undergoing more strenuous exercise merits further investigation.

The results of this study have practical implications. Alfalfa hay exhibited preventative or therapeutic capabilities of gastric ulcers in horses. Additional research is needed to better determine those constituents in alfalfa that contribute to a decreased severity of gastric ulcers compared to horses eating grasses. The minimal amount of alfalfa required to influence ulceration in horses needs to be evaluated. In certain areas of the United States where alfalfa is less available and more costly, horse owners would benefit economically by knowing how much alfalfa is needed for horses on a daily basis. Future work should also compare processed forms of alfalfa to determine if method of processing has any influence on the ability of alfalfa products to influence EGUS in horses. Little information is also available on the potential timing effects, more specifically, the role of alfalfa as influenced by feeding frequency or relative to the grain meal. Further work could also compare horses consuming similar diets in the presence and absence of forced exercise.

Relative to feeding CB hay, feeding alfalfa hay reduced ulcer severity scores in horses with gastric ulceration, and prevented ulcer development in 11/12 (92%) of horses fed alfalfa hay that did not have ulcers, whereas only 25% (3/12) of the horses without evidence of ulceration fed CB hay did not appear to develop ulcerations. Anti-ulcer medications can be exceedingly costly and for some owners the cost of this treatment precludes its use, particularly for purposes of preventing ulceration. Treatment of horses afflicted with EGUS with a current FDA-approved product (Gastrogard) costs horse owners up to $33.00/day or $990.00/month. The economical advantage of feeding alfalfa would be extremely beneficial. Given the frequency with which gastric ulceration occurs among horses used for various activities, there is great need for alternative or adjunctive strategies for managing this condition. Twenty-one observations of a combined 48 observations from the two trial periods were assigned ulcer severity scores of 2 or greater, which are possible candidates for treatment with anti-ulcer medication. Eleven of the 21 horses were fed alfalfa and all 11 lowered in ulcer score severity. If the 11 horses were given daily dosages of Gastrogard over a 28-day trial period the cost would be $10,164.00. Feeding alfalfa hay may represent a useful adjunct to anti-ulcer treatment for the control and prevention of EGUS.

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APPENDICES

APPENDIX 1. Physical measurements

Period 1 Body weights

Horse	Diet	Day	Weight (kg)
1	A	0	348.7
2	A	0	322.3
3	A	0	367.7
4	A	0	331.4
5	A	0	379.5
6	A	0	365.9
7	A	0	309.6
8	A	0	316.9
9	A	0	360.9
10	A	0	270.6
11	A	0	368.6
12	A	0	316.0
1	C	0	313.3
2	C	0	326.0
3	C	0	306.5
4	C	0	331.4
5	C	0	308.7
6	C	0	375.9
7	C	0	360.9
8	C	0	364.1
9	C	0	345.0
10	C	0	321.4
11	C	0	336.0
12	C	0	331.4
1	A	28	363.2
2	A	28	335.1
3	A	28	379.5
4	A	28	336.0
5	A	28	384.1
6	A	28	371.4
7	A	28	323.2
8	A	28	316.0
9	A	28	366.8
10	A	28	272.4
11	A	28	366.8
12	A	28	322.3
1	C	28	322.3
2	C	28	336.0
3	C	28	310.5
4	C	28	337.8
5	C	28	315.5
6	C	28	390.4
7	C	28	381.4
8	C	28	375.0
9	C	28	355.0
10	C	28	331.4
11	C	28	345.0
12	C	28	345.0

Period 2 Body weights

Horse	Diet	Day	Weight (kg)
1	A	0	331.4
2	A	0	338.2
3	A	0	320.1
4	A	0	345.0
5	A	0	326.9
6	A	0	381.4
7	A	0	360.9
8	A	0	365.5
9	A	0	354.1
10	A	0	
11	A	0	356.4
12	A	0	345.0
1	C	0	363.2
2	C	0	340.5
3	C	0	374.6
4	C	0	345.0
5	C	0	390.4
6	C	0	390.4
7	C	0	331.4
8	C	0	326.9
9	C	0	372.3
10	C	0	286.0
11	C	0	379.1
12	C	0	340.5
1	A	28	354.1
2	A	28	363.2
3	A	28	342.8
4	A	28	365.5
5	A	28	340.5
6	A	28	406.3
7	A	28	390.4
8	A	28	390.4
9	A	28	370.0
10	A	28	
11	A	28	372.3
12	A	28	374.6
1	C	28	380.5
2	C	28	356.4
3	C	28	397.3
4	C	28	360.9
5	C	28	410.9
6	C	28	401.8
7	C	28	345.0
8	C	28	342.8
9	C	28	370.0
10	C	28	295.1
11	C	28	390.4
12	C	28	354.1

APPENDIX 2A. Period 1 individual hay intake by diet (DM)

Horse	Coastal Diet (kg)	(g/kg BW)	Alfalfa Diet (kg)	(g/kg BW)
1	3.13	10.01	3.54	10.14
2	3.43	10.53	3.33	10.34
3	3.06	9.97	3.78	10.27
4	3.48	10.50	3.41	10.29
5	2.84	9.19	3.87	10.20
6	3.86	10.27	3.73	10.20
7	3.77	10.44	3.19	10.31
8	3.58	9.82	3.20	10.11
9	3.62	10.50	3.59	9.94
10	3.38	10.53	2.83	10.45
11	3.40	10.13	3.60	9.75
12	3.43	10.35	3.13	9.90
Total Mean	3.42	10.19	3.43	10.16

APPENDIX 2B. Period 1 individual concentrate intake by diet (DM)

Horse	Coastal Diet (kg)	(g/kg BW)	Alfalfa Diet (kg)	(g/kg BW)
1	3.35	10.69	3.69	10.60
2	3.45	10.58	3.43	10.65
3	3.26	10.65	3.91	10.62
4	3.53	10.66	3.51	10.58
5	3.30	10.70	4.03	10.63
6	4.00	10.63	3.90	10.65
7	3.82	10.60	3.31	10.68
8	3.87	10.62	3.32	10.49
9	3.69	10.71	3.83	10.62
10	3.42	10.65	2.91	10.75
11	3.54	10.53	3.92	10.63
12	3.53	10.66	3.30	10.44
Total Mean	3.56	10.64	3.59	10.61

APPENDIX 3A. Period 2 individual hay intake by diet (DM)

Horse	Coastal Diet (kg)	(g/kg BW)	Alfalfa Diet (kg)	(g/kg BW)
1	3.94	10.85	3.74	11.29
2	3.78	11.09	3.91	11.57
3	4.18	11.16	3.66	11.45
4	3.56	10.31	3.99	11.56
5	3.99	10.22	3.72	11.39
6	4.52	11.57	4.41	11.57
7	3.79	11.44	4.16	11.52
8	3.61	11.04	4.16	11.39
9	2.60	7.00	4.08	11.51
10	2.97	10.38		
11	3.81	10.06	4.08	11.45
12	3.80	11.16	3.99	11.57
Total Mean	3.71	10.52	3.99	11.48

APPENDIX 3B. Period 2 individual concentrate intake by diet (DM)

Horse	Coastal Diet (kg)	(g/kg BW)	Alfalfa Diet (kg)	(g/kg BW)
1	4.28	11.77	3.93	11.87
2	4.02	11.80	4.02	11.88
3	4.45	11.87	3.76	11.76
4	4.10	11.90	4.10	11.90
5	4.62	11.83	3.85	11.77
6	4.62	11.83	4.53	11.88
7	3.93	11.87	4.28	11.85
8	3.85	11.77	4.28	11.70
9	4.24	11.38	4.19	11.83
10	3.42	11.96		
11	4.45	11.73	4.19	11.76
12	4.02	11.80	4.10	11.90
Total Mean	4.17	11.79	4.11	11.83

APPENDIX 4A. Period 1 daily percent nutrient intake by hay (DM)

Nutrient	Coastal %	Alfalfa %
Protein	46.74	55.50
NDF	64.91	51.87
ADF	61.40	58.42
Calcium	31.89	59.98
Phosphorus	29.17	30.29
Magnesium	26.44	43.07
Potassium	63.15	67.84
Chloride	56.80	53.04
Copper	16.35	14.85
Iron	75.77	91.17
Manganese	41.12	31.07
Sodium	29.08	30.26
Sulfur	67.53	60.18
Zinc	18.86	13.65

APPENDIX 4B. Period 2 percent daily nutrient intake by hay (DM)

Nutrient	Coastal %	Alfalfa %
Protein	44.92	55.87
NDF	63.23	52.24
ADF	59.65	58.78
Calcium	30.32	60.34
Phosphorus	27.68	30.60
Magnesium	25.04	43.44
Potassium	61.43	68.17
Chloride	54.96	53.49
Copper	17.39	14.66
Iron	77.11	91.05
Manganese	42.94	30.75
Sodium	30.64	29.94
Sulfur	69.14	59.82
Zinc	20.03	13.47

APPENDIX 5. Gastric ulcer severity score

Horse	Diet	Day	Period	Score	Change	Diet	Day	Period	Score	Change
1	C	0	1	3	0	C	28	1	1	-2
2	C	0	1	2	0	C	28	1	1	-1
3	C	0	1	0	0	C	28	1	2	2
4	C	0	1	2	0	C	28	1	2	0
5	C	0	1	0	0	C	28	1	0	0
6	C	0	1	0	0	C	28	1	0	0
7	C	0	1	0	0	C	28	1	1	1
8	C	0	1	0	0	C	28	1	2	2
9	C	0	1	0	0	C	28	1	1	1
10	C	0	1	0	0	C	28	1	0	0
11	C	0	1	3	0	C	28	1	1	-2
12	C	0	1	0	0	C	28	1	2	2
1	A	0	1	0	0	A	28	1	0	0
2	A	0	1	0	0	A	28	1	0	0
3	A	0	1	0	0	A	28	1	0	0
4	A	0	1	0	0	A	28	1	0	0
5	A	0	1	0	0	A	28	1	0	0
6	A	0	1	2	0	A	28	1	0	-2
7	A	0	1	0	0	A	28	1	0	0
8	A	0	1	0	0	A	28	1	0	0
9	A	0	1	0	0	A	28	1	0	0
10	A	0	1	3	0	A	28	1	0	-3
11	A	0	1	3	0	A	28	1	1	-2
12	A	0	1	2	0	A	28	1	0	-2
1	A	0	2	2	0	A	28	2	0	-2
2	A	0	2	0	0	A	28	2	0	0
3	A	0	2	3	0	A	28	2	0	-3
4	A	0	2	3	0	A	28	2	0	-3
5	A	0	2	0	0	A	28	2	3	3
6	A	0	2	3	0	A	28	2	0	-3
7	A	0	2	0	0	A	28	2	0	0
8	A	0	2	2	0	A	28	2	0	-2
9	A	0	2	0	0	A	28	2	0	0
10	A	0	2	0	0	A	28	2		0
11	A	0	2	2	0	A	28	2	0	-2
12	A	0	2	2	0	A	28	2	0	-2
1	C	0	2	3	0	C	28	2	2	-1
2	C	0	2	2	0	C	28	2	2	0
3	C	0	2	0	0	C	28	2	2	2
4	C	0	2	3	0	C	28	2	2	-1
5	C	0	2	0	0	C	28	2	2	2
6	C	0	2	0	0	C	28	2	2	2
7	C	0	2	2	0	C	28	2	0	-2
8	C	0	2	1	0	C	28	2	2	1
9	C	0	2	0	0	C	28	2	3	3
10	C	0	2	2	0	C	28	2	2	0
11	C	0	2	1	0	C	28	2	3	2
12	C	0	2	2	0	C	28	2	2	0

APPENDIX 6A. Period 1 ulcer scores of CB fed horses

Horse	Day 0	Day 28	Change
1	3	1	-2
2	2	1	-1
3	0	2	2
4	2	2	0
5	0	0	0
6	0	0	0
7	0	1	1
8	0	2	2
9	0	1	1
10	0	0	0
11	3	1	-2
12	0	2	2
Mean	0.8	1.1	0.3

APPENDIX 6B. Period 1 ulcer scores of alfalfa hay fed horses

Horse	Day 0	Day 28	Change
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	2	0	-2
7	0	0	0
8	0	0	0
9	0	0	0
10	3	0	-3
11	3	1	-2
12	2	0	-2
Mean	0.8	0.1	-0.8

APPENDIX 7A. Period 2 ulcer scores of coastal hay fed horses

Horse	Day 0	Day 28	Change
1	3	2	-1
2	2	2	0
3	0	2	2
4	3	2	-1
5	0	2	2
6	0	2	2
7	2	0	-2
8	1	2	1
9	0	3	3
10	2	2	0
11	1	3	2
12	2	2	0
Mean	1.3	2.0	0.7

APPENDIX 7B. Period 2 ulcer scores of alfalfa hay fed horses

Horse	Day 0	Day 28	Change
1	2	0	-2
2	0	0	0
3	3	0	-3
4	3	0	-3
5	0	3	**3**
6	3	0	-3
7	0	0	0
8	2	0	-2
9	0	0	0
10			
11	2	0	-2
12	2	0	-2
Mean	1.5	0.3	-1.3

APPENDIX 8. Period 1 ulcer scores

Score	Coastal Diet Day 0	Coastal Diet Day 28	Alfalfa Diet Day 0	Alfalfa Diet Day 28
0	8	3	8	11
1	0	5	0	1
2	2	4	2	0
3	2	0	2	0
4	0	0	0	0

APPENDIX 9. Period 2 ulcer scores

Score	Coastal Diet Day 0	Coastal Diet Day 28	Alfalfa Diet Day 0	Alfalfa Diet Day 28
0	4	1	4	10
1	2	0	0	0
2	4	9	4	0
3	2	2	3	1
4	0	0	0	0

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