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Evaluation of Grazing Techniques as a Nutrition Source for Horses in a Pasture Environment

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EVALUATION OF GRAZING TECHNIQUES AS A NUTRITION SOURCE FOR HORSES IN A PASTURE ENVIRONMENT

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
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by
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ABSTRACT

Horses require daily access to forage in order to support their gastrointestinal tract function as well as natural grazing behaviors. Well-managed pasture provides horses with a consistent forage source and diminishes health risks such as colic, ulcers and stereotypies. However, equine grazing behaviors are more intense than other livestock and may be detrimental to plant and soil health. A grazing management technique specifically for horses is necessary to prevent both health and environmental issues. The following dissertation explores both the movement of required maintenance elements, such as feed, shelter, and water, as well as the manipulation of feeding frequency and mechanism to deliberately distribute equine activities within an equine pasture environment. Both considerations were evaluated via Global Positioning System units to determine location within respect to feed, shelter and water and scan-sampling to categorize grazing and non-grazing behaviors. It was determined that frequent movement of the feeding element may also distribute horse activities accordingly and become an efficient pasture management technique. Feeding frequency and mechanism was found to also distribute equine location with the most influential component being manual, twice daily feeding of a concentrated hay balancer.

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CHAPTER ONE

LITERATURE REVIEW

Pasture access is provided to horses not only as a source of nutrients but also exercise and other behavioral benefits (Bott et al., 2013). Horses have evolved as grazing animals, continuously consuming forage to support their digestive system. However, horse grazing behavior can create environmental risks for the land if not appropriately maintained. Thus, the primary goal of this literature review is to explore the current relationship between equids and forage as well as a potential remedies to aid the equine industry in opposing distinct grazing behaviors that are detrimental to the land.

Benefits of Forage to Horses

Horses are monogastric herbivores that receive the majority of their nutrients, if not all, from forage. Unlike other herbivores, horses are hindgut fermenters with a relatively small stomach designed to continuously consume forage (Janis, 1976). Their gastrointestinal tract (GIT) has evolved for efficient utilization of fiber from the diet as horses subsist on the structural component of the plant as opposed to reproductive portions like some other herbivores consume. Microbial fermentation of fiber in the hindgut can fulfill the majority of horses' energy requirements through the production and absorption of volatile fatty acids (VFAs). As the primary source of nutrients in an equine diet, forage provides not only energy but protein, fats, minerals, and vitamins in quantities sufficient enough to support a horse at maintenance or in light work (Hussein and Vogedes, 2003).

Forage, in the form of hay or pasture, is recommended to make up at least 50% of a mature horse's diet (NRC, 2007). While hay is used when fresh forage is limited, pasture provides horses with the opportunity to practice natural grazing behaviors and, simultaneously, obtain necessary nutrients. Intake can vary between 1.5 and 5.2% of their body weight in dry matter basis (DM), ranging highly on forage species, quality and availability. Pastures are commonly composed of a variety of forages with emphasis placed on a combination of legumes and grasses. Even though legumes are typically of higher quality (Ball et al., 2001; Catalano et al., 2015), the majority of pasture grasses are able to maintain horses at maintenance or in light work. If a pasture is well-maintained, grass yields can average 2 tons DM/hectare, which supplies adequate DM throughout the respective growing season for a 500 kg horse requiring 10 kg DM/day (NRC, 2007). Thus, the use of appropriate stocking rates and best pasture management practices are stressed to provide sufficient forage for horses to meet nutrient needs.

Allowing horses access to pasture, not only promotes proper GIT function and satisfies nutrient requirements but also provides numerous health and behavioral benefits as well. Horses in confinement are traditionally meal fed with minimal access to forage, potentially increasing their risk of chronic obstructive pulmonary disease, colic, gastric ulceration, and stereotypies (Bott et al., 2013). Houpt (1981) emphasized that the inhibition of grazing as a natural behavior can lead to stereotypical behaviors including circling, digging, kicking, wood chewing, swallowing air or self-mutilation. A survey by Christie et al. (2006) suggested a 12-hr increase in daily time spent on a grass pasture would halve the chances of horses having an oral stereotypy. Horses at pasture are also able to practice voluntary exercise, aiding in the bone development of growing horses (Bell et al., 2001).

Equine Grazing Behavior

Equine grazing behavior is multifaceted and influenced by several components including forage availability, plant composition and social interactions in addition to the climate and environment parameters. When not grazing, horses are observed standing or resting, moving freely, and drinking, which are categorized along with grazing and several other activities using ethograms (McDonnell, 2003). Ethograms are used to assess behavior, in that they aim to facilitate communication by categorizing known information and normalizing terminology on the topic (McDonnell, 2003). Similar to grazing, non-grazing behavior occurrences and duration are dependent upon changes in the environment including diet, accommodation, social interactions and sensory input (Ruckebusch, 1975). In relation to other livestock, horses differ in their physiology and preferences which have shaped their unique grazing and non-grazing behaviors while on pasture (Sharpe and Kenny, 2019).

Horses have dexterous lips and two sets of incisors capable of grazing forages close to the ground, whereas ruminants have only one set of incisors and rely on their tongues to retrieve food (Singer et al., 1999; Sharpe and Kenny, 2019). The lips and muzzle have tactile hairs and touch receptors to offer horses a detection mechanism to obtain desired forages. Their teeth are suited for grinding fibrous plant material and their large digestive tract efficiently extracts nutrients from the digesta to produce energy. In addition, equids have relatively narrower muzzles than large grazing ruminants (Janis and

Ehrhardt, 1987). Narrow muzzles compliment the horse's ability to graze closer to the ground and more frequent. Gordon (1989) observed ponies to remain on close-cropped grasslands, potentially because they were able to take deeper bites than cattle since ruminants do not have upper incisors.

Compared to ruminants, horses spend more time per day grazing, which is attributed to not only their prehension features but also their GIT. Horses tend to eat several smaller meals throughout the day more than likely due to the smaller size of the equine stomach as opposed to the multichambered rumen (Field and Taylor, 2012). Horses have adapted a periodic grazing behavior and have been observed to graze over 50% longer than cattle and over 60% of their daily time (Menard et al., 2002; Lopez et al., 2019; Fleurance et al., 2001). Arnold (1984) compared time budgets between cattle, sheep and horses whose grazing time ranged from 2.3-12.7, 4.4-10.6 and 4.1-16hr, respectively, dependent on forage availability. Grazing patterns of horses have also been investigated, with major feeding periods observed after dawn and before dusk (Mayes and Duncan, 1986; Arnold 1985). Mayes and Duncan (1986) determined free-ranging horses grazed 63-75% during daylight hours and 49-55% at night with frequency and duration of meals depending on season. Grazing duration and non-grazing activity are traditionally measured via visual observation for a designated period or through controlled grazing times (Martinson et al., 2017).

Horses are selective grazers and have strong preferences for certain forages over others. During grazing, horses continually move forward, tasting forages often and remaining on areas they seem to prefer (Archer, 1971). Thus, the length of grazing bouts is influenced by plant species, morphology, maturity and the quality of available forages (Krebs and Davis, 1993; Tyler, 1972). Fleurance and colleagues (2001) determined that horses prefer to graze shorter vegetation with improved quality over taller forage areas. Those authors indicated that horses chose plants based on their structure or growth stage, corroborating with other literature (McCann and Hoveland, 1991; Fleurance et al., 2010; DeBoer et al., 2017). McCann and Hoveland (1991) determined that maturity is an important factor in preference and palatability, whereby horses tend to choose the less mature forages with lower fiber content and higher nonstructural carbohydrates (NSC). Forage quality fluctuates daily, as plants accumulate NSC throughout the day to use them overnight. Non-structural carbohydrates are therefore lowest in the morning and highest in the afternoon after a day of sun access, impacting time of grazing (Ball et al., 2001). Grazing behavior may shift throughout the grazing season according to forage availability, day length and weather conditions. As desired forages decrease, animals tend to consume the less preferred plants in an attempt to maintain nutrient requirements regardless of the change in quality, i.e., lower digestibility and higher fiber content, decreasing intake in return (Osoro et al., 2012). Fiber content may be measured through hand-harvesting forage samples and completing subsequent laboratory analysis (Martinson et al, 2017).

Equine Grazing Issues and Management Techniques

Despite the benefits that pasture may provide to animals, horses can cause permanent damage to plant structure largely due to close-cropping grazing time in a limited space, as well as plant type, climate, and soil fertility. Forage preferences affect the even utilization of forage as well as plant persistence if desired species are repeatedly grazed (Marten, 1978). Horses overgraze desired forage areas below recovery level, leaving undesired plant species to grow and mature. In addition, pasture forage can be impacted up to 90% via trampling, urination, or defecation due to their high activity and hooves (Carson and Wood-Gush, 1983; Sharpe and Kenny, 2019). If not managed appropriately, negative implications such as movement of sediment, nutrients, and pathogens into nearby water sources can occur (Bott et al., 2013).

Current equine pasture management techniques are supported by data from other grazing species with minimal knowledge on equine-specific systems. Selection of grazing technique is commonly dependent upon the potential for pasture production to offset labor and equipment costs. Several grazing systems have been designed to improve forage productivity and quality as well as environmental stewardship, with the two most common being continuous and rotational grazing.

Continuous grazing is a low-maintenance method allowing animals unlimited pasture access without rest during the growing seasons. Thus, continuous grazing leads to underutilization of forage as horses prefer to graze immature, growing plants and avoid the mature areas. With the overgrazing of immature forages, the nutritional value of pastures will continually decrease as only the mature plants are available. The overgrazing of desired forages in a continuous system also results in reduced plant health and vigor, requiring additional time to recover. In addition, continuous grazing systems are predisposed to uneven manure deposition, leading to an excess of nutrients in specific pasture areas (Henning et al., 2000). Alternative techniques have therefore been developed to decrease risk to pasture health and promote benefits for horses.

Rotational grazing is often recommended as an alternative to the traditional continuous method due to economic and environmental advantages (Weinert and Williams, 2018). A rotational system encompasses animals moving between multiple grazing areas, allowing each a rest and regrowth period to maximize forage production (Bott et al., 2013). During the rest and regrowth period, forages are given the advantage to build energy stores and root reserves without being continuously depleted. While in a rotational system, horses are not given the opportunity to overgraze desired forages allowing for consistent plant growth and usage. Rotational grazing systems have been shown to improve pasture productivity and nutrient content, providing horses with a consistent source and quality of forage while also alleviating the environmental impacts of continuous grazing. Webb and colleagues (2009) compared continuous versus rotational grazing by horses, in which the rotational system resulted in more available forage, prolonged grazing with less detrimental effects on the forage stand, and assistance in the prevention of run-off. Weinert and Williams (2018) evaluated recovery rate of forages between continuous and rotational grazing techniques and determined differences in yield throughout much of the growing season. More specifically, a total of nine months of rest was necessary for herbage mass in the continuous pasture to reach similar levels as the rotational pastures.

Despite the potential forage and environmental benefits of rotational grazing, lack of resources may complicate the implementation by horse farm owners as assessed through multiple surveys. Singer and colleagues (2002) distributed a survey to evaluate pasture management practices of equine property owners in New Jersey. Those authors determined 54% of managers employed rotational grazing, yet managers did not necessarily follow the recommended stocking density. In Pennsylvania, a higher percentage (65%) of equine farm owners reported using a rotational grazing system compared to continuous grazing (35%; Swinker et al., 2011). However, only 24% allowed pasture to regrow to the recommended grazing height and 45% reported occasionally resting pastures. Similarly, a Maryland survey determined less than one third of horse farm owners used rotational grazing and always allowed pastures to recover despite owners declaring to have "very high" knowledge of stocking density and rotational grazing techniques (Fiorellino et al., 2013). A follow-up trial was conducted in which equine facilities were visited to evaluate pasture management practices (Fiorellino et al., 2014). The authors discovered a high level of vegetative cover supporting the 21% of owners who reported to use rotational grazing, but the moderate sward height, minimal use of sacrifice lots and high occurrence of erosion implied the correct use of grazing techniques were not fully employed. The inconsistencies within these surveys further indicate horse farm owners need other user-friendly pasture management techniques designed for equids.

Required Maintenance Elements for Horses

Pasture management techniques established for other livestock animals are still utilized for horses even though their grazing behavior is irregular, justifying the need for establishing improved management techniques catered towards horse grazing behavior.

Within pasture environments, required maintenance elements, such as water, supplemental food, and shelter, are not evenly distributed throughout a pasture, causing horses to focus their activities around the most limited resource and creating the risk of overgrazing (Ganskopp, 2001). High traffic areas are also subject to high occurrences of ice, mud and nutrient loss via water run-off as forage stand is minimal, increasing risks to both horses and environment.

Water

Depending on environmental conditions, among other factors, horses require 20 to 76 liters of water daily. Drinking frequency and time of day can occur with great variation, increasing foot traffic around the water source (Hinton, 1978). A study investigating the feeding and drinking behavior of mares and foals determined that the frequency of drinking was directly correlated to ambient temperature, with a large increase in occurrence when temperatures rose above 30°C (Crowell-Davis et al.,1985). Crowell-Davis and colleagues also reported that the distribution of drinking bouts occurred most frequently in the mid-afternoon and were lowest in the morning (1300-1700 and 0900 to 1300, respectively). Even when not utilizing water resources, Scheibe and others (1998) found Prezwalski horses, maintained in a semi-reserve environment for over a year, remained near water troughs for extended periods of time and rested in its vicinity.

Supplemental feed

The quality of pasture is typically more than sufficient for adult horses at maintenance when provided unlimited access. However, factors including but not limited to environmental conditions, forage health, maturity and plant type may inhibit pasture quality justifying the use of supplemental feed. Supplemental feed, such as a ration balancer, is formulated to provide horses with nutrients that are lacking within pasture (Rensia, 2010). Forage with an adequate amount of energy may not have similar levels of protein, vitamins and minerals required for the physiological activity of a horse. Ration balancer is efficient in providing a concentrated amount of crude protein, vitamins and minerals to meet horse requirements while not overfeeding energy that may already be provided by the forage.

Common pasture for horses consisting of several cool and warm season provide an appropriate amount of crude protein (CP) for a mature horse at maintenance. Allen and others (2013) investigated the nutritive value of several cool-season grasses within equine pastures over a two-year timeline with emphasis placed on CP concentration, amongst others. The authors found that forages with even the lowest concentration of CP (192 g) provided more than twice the amount of CP needed by a mature horse in moderate exercise as described by the 2007 National Research Council (NRC). Within the aforementioned study, it should be noted that CP concentrations differed among grasses in both trial years indicating concentrations have the potential to decrease with maturity. A previous study showed a similar decrease in CP, as well as an increase in neutral detergent fiber (NDF) and acid detergent lignin (ADL), as multiple species of forage matured (Hockensmith et al., 1996). Thus, supplemental feed may be warranted as/if pasture forage matures, because decreasing nutrients would be available to the horse in this mature forage.

Pasture is notoriously variable in essential trace minerals causing a potential imbalance and need for supplementation. For example, Chelliah and colleagues (2008) evaluated trace mineral concentrations of annual cool season forages grazed by beef cattle in North Florida over multiple growing seasons. Those authors concluded that their forage samples had deficient concentrations of Cu, Co and Se and recommended the inclusion of adequate mineral mixture rates to growing beef cattle. Although the previous study indicated pasture mineral quantity for cattle, reports on the nutritive value of pasture for horses regarding mineral requirements have also been conducted. A review by Hoskin and Gee (2004) noted up to 30% of New Zealand farmland is deficient in Se and supplementation is advised to diminish the risks of white muscle disease in adult horses or decreased growth rate in foals. Bott and others (2013) noted that pasture can be missing appropriate levels of Na, Cl, Cu, and Zn, which further emphasizes the need of supplemental feed rich in these minerals for horses on pasture of varying soil quality.

Shelter

Horses, along with other livestock, are most productive within a thermal neutral zone (TNZ) of -15 to 25ºC. Thus, horses have adapted to cooler climates and tend to suffer metabolic issues with temperatures above 25ºC (NRC, 2007; Hristov et al., 2018). If temperatures are below the TNZ, feed intake will increase to meet increased maintenance energy needs. Heat stress, on the other hand, may lead to decreased feed intake amongst other production tasks when temperatures are above the TNZ. Thus, shelter is highly recommended by numerous best practice guidelines to protect horses against adverse conditions as shade access affects the horse’s physiological homeostasis

(Snoeks et al., 2015). Holcomb and colleagues (2014) observed individually housed horses, and determined that horses without access to shade in a hot, sunny environment had higher rectal and skin temperatures and higher respiration rates than completely shaded horses.

Extensive research has shown that horses seek shelter during more extreme weather such as rainy, windy, hot and/or sunny days, with shelter need varying per region and breed. Snoeks and colleagues (2015) found domestic horses used shelter approximately half of the observed time, with increased values seen in cold and hot temperatures. A previous trial investigated daytime shelter-seeking behavior in domestic horses and determined the horses utilized shelter more frequently in rainy, breezy, snowy, and cooler temperature conditions than in moderate weather conditions (Heleski and Murtazashvili, 2010). Those authors also proposed that type of shelter, as well as its isolation, ventilation, and orientation, could affect the horses' decision to use the element. An additional factor that has been shown to influence shelter-seeking behavior by horses is feed availability. Heleski and Murtazashvili (2010) emphasized that if forage quality was adequate, horses would continue to graze or eat hay instead of seeking shelter, despite inclement weather. Horses have also even sought out shelter to avoid pest harassment. Previous trials have suggested that horses perform comfort behaviors such as tail swishing and head shaking in addition to seeking shelter to avoid insects, specifically in warmer temperatures (Heleski and Murtazashvili, 2010; Snoeks et al., 2015). Best management practices recommend the inclusion of shelter to avoid negative biological or behavioral responses caused within pasture-housing environments.

Maneuvering Required Maintenance Elements

Feed, Shelter, Water

Within livestock pasture environments, maintenance elements such as water, supplemental food, shelter and resting areas are not evenly distributed, causing the risk of overgrazing by focusing their activities around required resources (Ganskopp, 2001). Prior studies have evaluated configurations of required maintenance elements to manipulate the distribution of cattle, but to this author's knowledge this concept has been minimally investigated in horses. Ganskopp (2001) found that altering the position of water shifted cattle activity location, as cattle remained near the water, while Bailey and Welling (1991) concluded cattle can be lured with a dehydrated molasses supplement to improve uniformity of grazing underutilized rangeland. It should be noted that pastures evaluated in these cattle studies were much larger (>800 ha) than traditional equine pastures, however, the impact required element placement has on grazing behavior and location of cattle may also have relevance in equine grazing management. For instance, a survey investigating best management practices in Maryland noted the movement of portable feeders to different locations within pastures to correct soil erosion (Fiorellino et al., 2014). Another trial investigating group housing behavior recommended the distance between elements such as feed and water be as long as possible to motivate horses to distribute their movement (Hoffman et al., 2012). Thus, further investigation is warranted to determine the impact that manipulating required maintenance elements has on equine grazing location and behavior within a pasture environment.

Feeding frequency

Horses have expressed a drive to consume supplemental feedstuffs whether fed alone or in conjunction with forage. Henneke and Callahan (2009) reported high intake of *ad libitum* concentrate when horses were simultaneously offered *ad libitum* access to hay. While the previous study highlighted *ad libitum* concentrate access, traditional supplemental feeding practices largely revolve around ease of management; thus, horses are commonly fed twice a day (Harris, 1999). However, previous research has also explored the effects that multiple daily feedings have on horse health and behavior (Clarke et al., 1990; Cooper et al., 2004). Concentrate meals have been associated with increased risks of colic (Tinker et al., 1997), ulceration (Nicol et al., 2002) and stereotypies (Nicol, 1999). Horses are naturally trickle feeders; therefore, dividing supplemental feed rations into smaller more frequent meals may support horse's GIT function and decrease unwanted feeding behaviors. Cooper and colleagues (2004) increased the frequency of concentrate meals to investigate the behavior of stabled horses, which resulted in a decrease of oral stereotypies and more time taken to consume meals. Despite the decrease in oral stereotypies, the increase in meal frequency did influence pre-feeding activities such as nodding and weaving, which may be potentially due to pre-feeding cues made by humans. Since meal feeding practices typically apply to stabled horses, further exploration is needed to determine the effects they may have on those at pasture.

Feeding mechanism

Since providing multiple meals a day to horses adds labor for owners and a potential increase in pre-feeding stereotypies, the use of automatic feeders has been considered. Hoffman and colleagues (2012) employed an automatic concentrate feeding station (CFS) to examine horse behavior and distribute activity within a group yard-housing system. Horses adapted to the CFS quickly and their implementation of small meals further enhanced horse movement and lying down behaviors as opposed to within traditional individual stalls. Frölich and colleagues (2004) evaluated a similar CFS to provide an optimal management technique for group-housed horses. Those authors determined the automatic feeders efficiently dispensed several meals throughout the day in a controlled manor, reduced food rivalry, and increased activity, similarly to the prior (Hoffman et al., 2012) trial. It should be noted each CFS within these previous studies utilized identification sensors to allow all horses equal access to a consistent amount of feed despite hierarchy. However, similar to increasing quantity of meals, automatic feeders have not been assessed within an equine pasture environment and requires investigation before recommendation as an efficient grazing management technique.

Summary

Horses require daily access to forage to support GIT function as well as natural grazing behaviors. Well-managed pasture provides horses with a consistent forage source and diminishes health risks such as colic, ulcers and stereotypies. However, equine grazing behaviors are more intense than other livestock and may be detrimental to plant and soil health. A grazing management technique specifically for horses is necessary to combat both health and environmental issues. The movement of required maintenance elements within a pasture has been explored in cattle and may be relevant in equine grazing management. In addition to movement of feed, shelter, and water, supplemental feeding frequency and mechanism may aid in the distribution of equine grazing behaviors and location. The remaining chapters that follow discuss the implementation of required maintenance elements within equine pastures and their efficacy as an equine grazing management technique for the horse industry.

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CHAPTER TWO

MANEUVERING REQUIRED MAINTENANCE ELEMENTS TO MONITOR THE LOCATION AND BEHAVIORS OF HORSES IN A PASTURE ENVIRONMENT

Abstract

Required maintenance elements such feed, shelter and water are not evenly distributed within pasture environments, leading horses to focus their activities around concentrated resources and creating the potential risk of overgrazing. The objectives of this study were to determine if 1) varying positions of required elements including feed (F), shelter (S) and water (W) affected horse presence within 23 m (P23) of required elements and 2) placement of required elements had an effect on the grazing distribution and behavior of horses. It was hypothesized that both grazing location and behaviors within a pasture would be affected by altering position of F, S, and W. Six mature mares were paired and assigned to graze three element configurations tested in duplicate (CONF1-A, CONF1-B; CONF2-A, CONF2-B; CONF3-A, CONF3-B) of F, S, and W within six individual pasture plots in an incomplete randomize design. Individual pairs grazed one of six pasture plots for four 7 d periods with 72-hr washouts between each period. Horses were fed a hay balancer to manufacturer's recommendation (0.23 kg of Nutrena® Empower® Topline Balancer, Cargill Incorporated©, Minneapolis, MN) at 0715 and 1715 daily with *ad libitum* access to water. Horses were fitted with global positioning systems (GPS; Trak-4 GPS Tracker, Pryor, OK), logging measurements every 10 min. Horses were also visually observed for three 2-hr intervals (MOR; NOON; EVE) daily during the 7-d grazing periods, with behaviors recorded every 5 min via scan-sampling. Trained observers classified horse behavior as either grazing or other activity. Linear mixed

models were developed that related occurrence of behaviors or horse presence within 23 m to fixed effects of CONF and element; the random effects of period, activity, time, day and horse; and interactions. An ANOVA was used to determine if the fixed effects were significant, followed by Fisher's Protected LSD to compare means. There was an effect of element on P23 ($P < 0.01$), as hypothesized, with F being most influential ($P < 0.05$) in that horses spent the most time within P23 for F in comparison to S and W. Horses spent more time grazing ($P < 0.05$) than other observed behaviors, regardless of CONF, followed by standing/resting, free movement and eating grain. Moving feeding location frequently may alter grazing location, thus distributing animal concentration accordingly and decreasing the risk of overgrazing. Therefore, future studies investigating moving feed only may illuminate new methods of pasture management.

Introduction

Horses meet the majority of their caloric requirements through forage, traditionally offered in forms of harvested hay or fresh forage. Allowing horses access to fresh forage, or pasture, provides not only a source of nutrients but numerous behavioral and health benefits as well. Such benefits include reduced risk of colic, gastric ulcers, cribbing and growth-related issues in young horses (Martinson et al., 2016). Equine grazing behavior is complex and influenced by several variables including not only nutrition but also plant composition, forage availability, social interactions, weather and other environmental variables including access to shade (Krebs and Davis, 1993). Despite the benefits that pasture access provides, horses' unique behavior of grazing desired forage to a shorter height and more frequently than other livestock species causes multiple stressors to the land. Due to the grazing of preferred plants, horses tend to damage plant integrity and create environmental concerns such as soil compaction and water pollution from run-off (Bott et al., 2013). Pasture management techniques established for other livestock animals are still utilized for horses even though their grazing behavior is significantly different, justifying the need for establishing improved management techniques catered towards horses.

Within livestock pasture environments, maintenance elements such as water, supplemental food, shelter and resting areas are not evenly distributed, causing the risk of overgrazing by focusing their activities around required resources (Ganskopp, 2001). Prior studies have evaluated configurations of required maintenance elements to manipulate the distribution of cattle, but to the authors' knowledge this concept has not yet been investigated in horses. Ganskopp (2001) found that altering the position of water shifted cattle activity location, as cattle remained near the water, while Bailey and Welling (1999) concluded cattle can be lured with a dehydrated molasses supplement to improve uniformity of grazing underutilized rangeland. Previous literature investigating the impact required element placement has on grazing behavior and location of cattle may also have relevance in equine grazing management. Depending on environmental conditions, among other factors, horses require 20 to 76 liters of water daily in which drinking frequency can occur several times, increasing foot traffic around the water source (Crowell-Davis et al., 1985). Use of shelter is also dependent on environmental conditions. Literature has shown horses seek shelter during more extreme weather such as rainy, windy, hot and/or sunny days, with need varying by region (Snoeks et al., 2015). Supplemental feed may also be necessary for horses depending on stage of life as well as pasture health and yield; concentrate is typically provided at a minimum of two meals daily. Horses are therefore prone to spend ample amount of time in the above areas, negatively impacting soil and forage condition. Thus, the movement of required elements may provide equine managers with an efficient technique to minimize the concentration of grazing in certain pasture areas and thus lessen potential detrimental impacts of overgrazing in these areas. The objectives of this study were to determine if 1) varying positions of required elements including feed, shelter and water affect horse presence near required elements and 2) placement of required elements had an effect on the grazing behavior of horses. It was hypothesized that both grazing location and behaviors within a pasture would be affected by altering position of feed, shelter, and water.

Materials and Methods

This research was approved by the Institutional Animal Care and Use Committee of Clemson University (IACUC Protocol #: 2020-037).

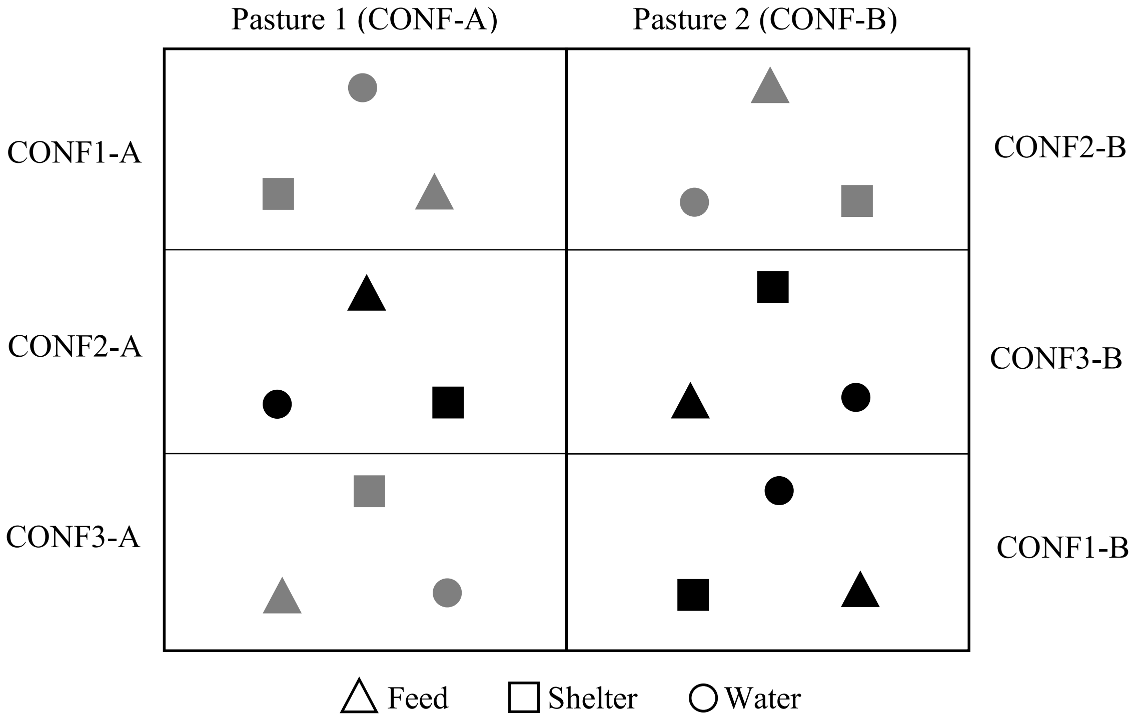
Animals and Environment

This research study was conducted at the Clemson University Equine Center in Pendleton, SC. The trial lasted for 40 days, beginning the 28th of September and ending November 6th of 2020. Horses grazed six pasture plots approximately 0.95 ha in size that were mowed to a sward height of approximately 20 cm prior to grazing. Five mature American Quarter Horse and one Warmblood mares (12.7 ± 2.9 yr, 500 ± 12.4 kg) underwent grazing at a stocking rate of 0.47 horses per ha (Bott et al., 2013). The pasture stand has not been renovated in over ten years prior to the current trial with no fertilization or seeding, and thus forage composition reflects that of past establishment. The soil in all pasture plots consisted of Cecil sandy loam with approximately 80% at a slope of 2-6% and the remaining at 6-10%. Horses had the majority of a free line of sight to horses grazing in other pasture plots with less than 10% of CONF3-A not visible by the remaining CONF. Climate measurements were also acquired from the National Weather Service throughout the course of the trial, with an average temperature of 17.2°C, range of -0.56°C to 28.9°C, and average precipitation of 3.3 ± 1.27 mm.

Experimental Design

Horses were paired and assigned to graze three element configurations (CONF) of feed (F), shelter (S) and water (W) within two pastures divided into three plots each. This resulted in six adjoining pasture plots (0.95 ha each) grazed in an incomplete randomized block design. The six pasture plots utilized in this study were randomly assigned to one of the CONF such that each CONF was replicated in two plots (Fig. 2.1). Plots were defined using electric 38 mm polytape (Pasture Management Systems®, Inc., Mt. Pleasant, NC). A pair of horses was randomly assigned to one of the CONF and grazed within that pasture plot for 7 d. Three pasture plots were grazed simultaneously, each by a different pair of horses within one 7-d period. To ensure pasture forage availability and CONF replication, the trial consisted of four 7-d periods, subsequently referred to as Periods 1-4, and four preceding 72-hr washout phases. For instance, CONF1-B, CONF2-A and CONF3-B were grazed in Periods 1 and 3 while the remaining CONF were grazed in Periods 2 and 4. Each period was initiated with a 72-hr washout in which horses were placed in individual outdoor stalls with no pasture access. During washout periods, horses were fed *ad libitum* long-stem forage along with a concentrate hay balancer fed to manufacturer's recommendation twice daily at 0715 and 1615 (0.23 kg of Nutrena® Empower® Topline Balancer, Cargill Incorporated©, Minneapolis, MN). While in the pasture plots, horses were also fed concentrate hay balancer (0.23 kg) twice daily at 0715 and 1615. Shelters were portable man-made structures with canvas tops, in which horses had a one-week adjustment period to pre-trial. Water was provided *ad libitum* in portable 100-gallon stock tanks.

Figure 2.1. Schematic diagram of pastures 1 and 2, denoted by CONF-A or -B, were divided into three plots (0.95 ha each) to make six adjoining pasture plots. Three element CONF were grazed by two horses each simultaneously within each Period (total of 6 horses grazed per Period). CONF1-B, CONF2-A and CONF3-B were grazed in Periods 1 and 3 (black) while the remaining CONF were grazed in Periods 2 and 4 (grey). Each Period lasted for 7 grazing days, in the months of October and November.



Pasture Sampling and Analysis

Prior to the start of each Period, forage composition and quality were determined through collection of ten samples from each pasture plot. Pasture composition was visually assessed using the double DAFOR scale in which the relative abundance of forage and weed species within a 0.5-m^2 quadrat were measured (Abaye et al., 1997; Virostek et al., 2015). Forage species that covered >75% of the area assessed were assigned “dominant” (D); “abundant” (A) to species that covered 50-75%; “frequent” (F) to species that covered <50% but were well distributed in the area; “occasional” (O) species were those found a few times; and “rare” (R) are species that only occurred one or two times in the given area. Post-composition analysis, forage within the 0.5-m^2 quadrat were collected via hand-clippings to ground level and subsequently dried at 55°C for 48 hr in a forced-air oven (Weinert and Williams, 2018). Dry samples were ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Ground samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) content. Neutral detergent fiber and ADF concentrations were determined using an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NW). Sodium sulfite and α-amylase (Sigma no. A3306: Sigma Chemical CO., St. Louis, MO) according to Van Soest and colleagues (1991) were included for NDF analysis.

Behavior Sampling

During the grazing periods, horses were fitted with a Global Positioning System (GPS) unit (Trak-4 GPS Tracker, Pryor, OK) mounted onto individual identification collars (Martinson et al., 2016). Horses carried collars for a one-week adjustment period prior to the study (Ganskopp, 2001). GPS units remained mounted on the upper neck of horses for all 7-d grazing periods, logging location measurements every 10 min, thus producing an expected 4128 recorded positions per horse. The GPS response variable included frequency of horses present within 23 m (P23) in relation to elements. The 23-m distance was utilized due to being the halfway point between elements (Ganskopp, 2001).

Horses were live observed for three, 2-hr timepoints (0700-0900; 1200-1400; 1700-1900) per day throughout all 7 d of each period. Horses were conditioned to their designated pasture plot 12 hr before the first observation of each Period began. Activity was recorded using the scan sampling method (Altman, 1974), where a 5-s scan of the horses was made every 5 min and the activity of each individual was recorded. Two individuals from the same set of observers throughout the trial were randomly assigned to each three, 2-hr timepoint to both observe and concur all horse behavior within all pasture plots. Horse behavior was classified as either grazing (actively consuming pasture forage) or non-grazing activity, otherwise recorded as free movement, drinking, standing/resting, social interaction, biting at flies/insects, lying down/rolling, eating grain, or licking salt block.

Statistical Analyses

A linear model was developed that related forage composition to the fixed effects of pasture plots and period; and interactions. Another linear model was developed that related forage quality to fixed effects pasture plots and period; and interactions. A linear mixed model was established that related horse presence within 23 m to the fixed effect of element; the random effect of configuration, horse, day and period; and interactions. A final linear mixed model was developed that related the frequency of activity to the fixed effect of behavior; the random effects of period, configuration, time, day and horse; and interactions.

An ANOVA was used to determine if the fixed effects were significant. If the fixed effects were found to be significant, then Fisher's Protected Least Significant Difference was used to compare the means. All statistical analyses were completed using JMP version 15 (2019 SAS Institute Inc.). Data are presented as LSM ± SEM and P-values less than 0.05 were considered evidence of statistical significance. Examination of residuals plots combined with tests (Shapiro-Wilk and Levene) were used to assess ANOVA assumptions concerning normality and stable variance. ANOVA independence assumptions were addressed by including all possible factors (that could possibly lead to clustering and correlation of observations) in the linear mixed models.

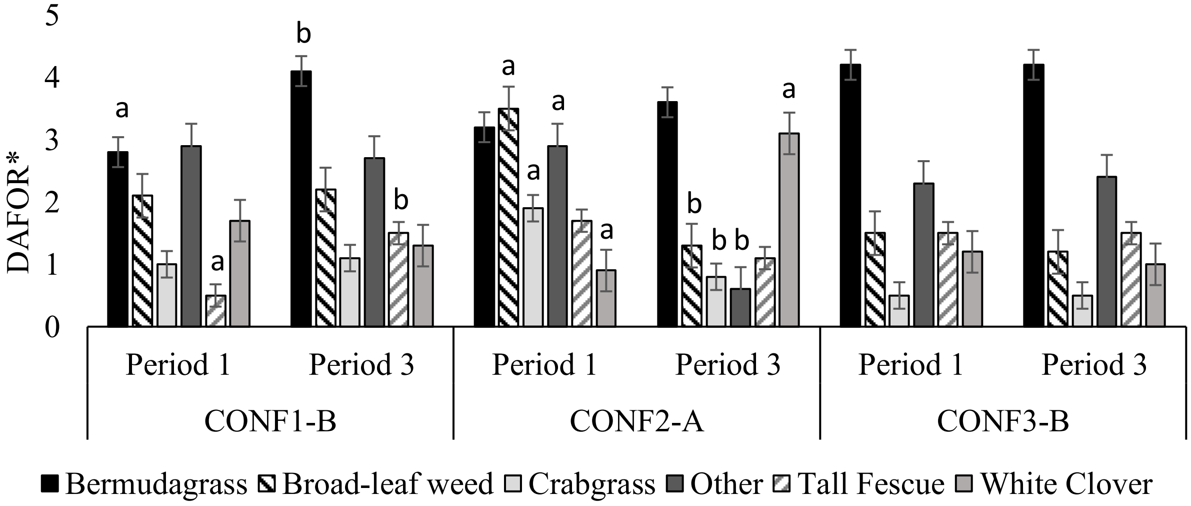
Results

Pasture

When evaluating composition, a total of five plant species were found within each of the six pasture plots, including Bermudagrass (*Cynodan dactylon*), broad leaf weed, Crabgrass (*Digitaria sanguinalis*), Tall Fescue (*Schedonorus pheonix*), White Clover (*Trifolium repens*), and dead material or bare ground categorized as 'Other' (Fig. 2.2; Fig. 2.3). All species were found during each period and pasture plot with the exception of Crabgrass in Period 2 CONF2-B and CONF3-A and Tall Fescue in Period 2 CONF1-A. Some differences in species abundance were seen between periods and CONF within pasture plots. An increase in both Bermudagrass and Tall Fescue abundance were observed from Period 1 to 3 in CONF1-B ($P < 0.05$). White Clover also increased between Period 1 to 3 in CONF2-A ($P < 0.05$), whereas the amount of broad leaf weed, Crabgrass and 'Other' decreased ($P < 0.05$). No forage composition differences were observed in CONF3-B between Periods 1 and 3. Within Periods 2 and 4, Tall Fescue occurrence in CONF1-A increased, but decreased in CONF3-A. Also, in CONF3-A, there was an increase in White Clover between Period 2 and 4. A difference in 'Other' also occurred in CONF2-B, decreasing from Period 2 to 4.

Mean NDF and ADF values varied among forages across periods within pasture plots (Table 2.1). Between Periods 1 and 3, ADF values increased from plot to plot, i.e., 41.3% to 45.2% in CONF1-B, 41.4% to 46.5% in CONF3-B and 36.4% to 42.9% ± 0.63 in CONF2-A (Period 1 to 3; $P < 0.05$). Neutral detergent fiber remained mostly consistent with only a single decrease in CONF3-A from Period 2 to 4 (89.6% to 67.6% ± 6.9; $P < 0.05$).

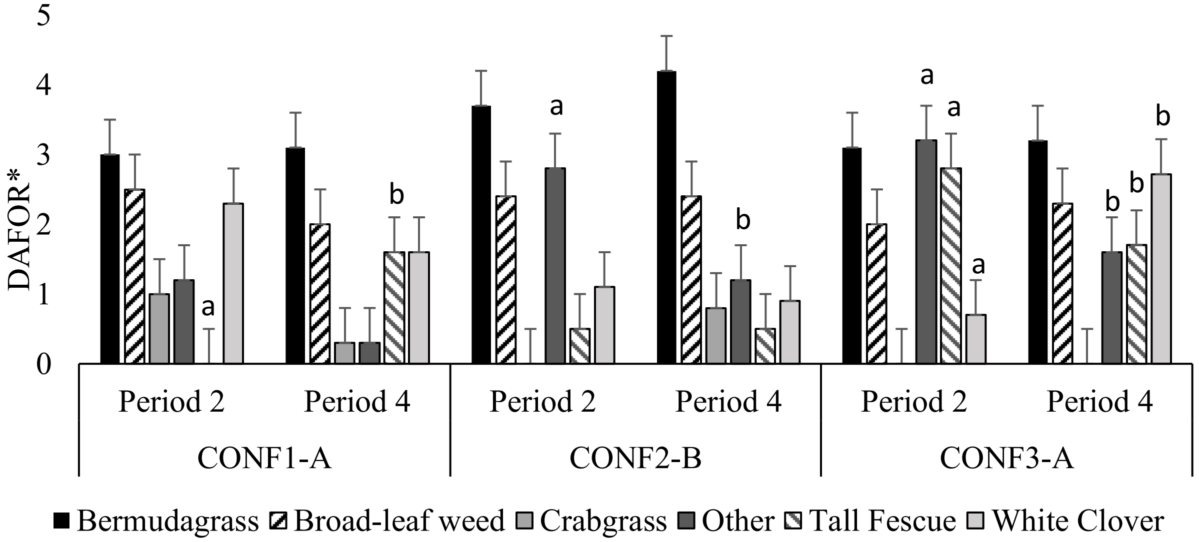
Figure 2.2 Comparison within element CONF (CONF1-B; CONF2-A; CONF3-B) across Periods (1 and 3); showing differences in forage composition, via the double DAFOR scale; D=5, A= 4, F=3, O=2, and R=1. Data are presented as LSM with SEM error bars.



*Standard error of all LSM were 0.56.

[ab] Identical forage species across Periods 1 and 3 within one replicate of each of the three plot CONF not connected by the same letter are significantly different ($P < 0.05$).

Figure 2.3 Comparison within element CONF (CONF1-A; CONF2-B; CONF3-A) across Periods (2 and 4); showing differences in forage composition, via the double DAFOR scale; D=5, A= 4, F=3, O=2, and R=1. Data are presented as LSM with SEM error bars.



*Standard error of all LSM were 0.56.

^{ab} Identical forage species across Periods 2 and 4 within one replicate of each of the three plot CONF not connected by the same letter are significantly different (*P* < 0.05).

Table 2.1 Nutritive values of forage available by Period within pasture plots of repeated CONF, in which Periods 1 and 3 had identical CONF as did Periods 2 and 4. Data are presented as percent LSM ± SEM.

	Plot	NDF (%)*	ADF (%)*
Period 1	CONF2-A	77.0^{a}	39.4^{a}
	CONF1-B	73.4^{a}	41.3^{a}
	CONF3-B	69.4^{a}	41.4^{a}
Period 3	CONF2-A	67.6^{a}	42.9^{b}
	CONF1-B	69.5^{a}	45.2^{b}
	CONF3-B	53.1^{a}	46.5^{b}
Period 2	CONF1-A	75.3^{a}	47.9^{a}
	CONF3-A	89.6^{a}	48.4^{a}
	CONF2-B	74.1^{a}	53.3^{a}
Period 4	CONF1-A	67.4^{a}	48.6^{b}
	CONF3-A	67.6^{b}	45.6^{b}
	CONF2-B	70.1^{a}	54.2^{a}

*Standard error of all LSM were 6.9 and 0.63, respectively.

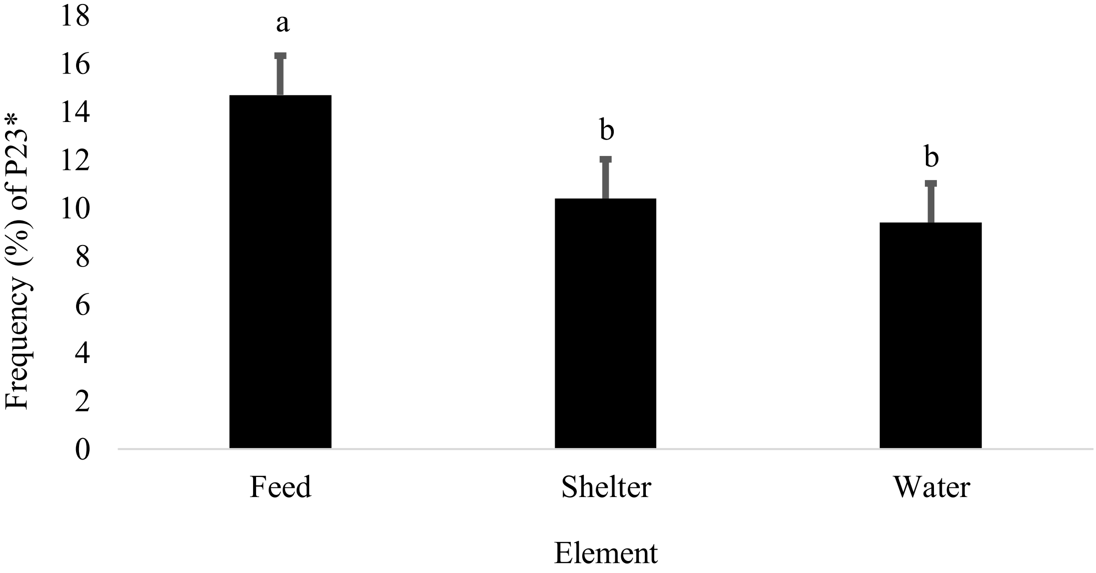
^{ab}Values with differing letters within rows of repeated CONF are significantly different (*P* < 0.05).

Pasture Location via GPS

Over the 28 days treatments were in effect, each GPS unit was expected to record 144 positions daily and 4,032 total. The Trak-4 GPS units contained hardware and logical processing to calculate position based on GPS satellite signals, tracking location by user-selected time interval and movement of unit, potentially producing more or less than the expected number of positions. Four of the six units either reached or exceeded the expected number of positions, delivering an average of 4,128 ± 40.9. The remaining two units generated 97.7 and 98.1%, respectively, of the expected positions. Thus, results were calculated based on the daily expected number of positions.

All six horses were located within 23m of all three elements totaling 22.7 to 29.6% of the overall GPS positions recorded. Element did have an effect on horse presence within 23m, with concentrate feeding area being the most frequented ($P < 0.05$) followed by water, then shelter (14.7%, 10.4% and 9.4%, ± 0.37, respectively; Fig. 2.4). An interaction between CONF, Element and Period also had an effect on P23 ($P < 0.05$; Table 2.2), in which CONF2-B contained the highest recording of locations of horses within 23m (32.6 ± 1.6%) in Period 2, of the concentrate feeding area, followed by CONF 3-A in Period 2 and 4 (21.6% and 20.6% ± 1.6, respectively).

Figure 2.4. Daily frequency of horse presence within 23m (P23) each element; feed, shelter, and water. Data are presented as LSM with SEM error bars.



*Standard error of all LSM was 0.37.
^{ab}Element not connected by the same letter are significantly different ($P < 0.05$)

Behavior

Observers monitored the behavior of the six mares for a total of 168 h, with grazing activity averaging 76.9% daily. Thus, grazing activity was the most observed behavior ($P < 0.05$; Table 2.3). Behavior did vary within pasture plots and Period, in which CONF3-A yielded the most grazing ($P < 0.01$) followed by CONF2-B, both in Period 4. No difference in grazing activity was observed between the three observation times (MOR; NOON; EVE).

Table 2.2 Percent of time horses spent 23m from each element on a daily basis within each of the six CONF. Data are presented as LSM.

			Day							
Period	Treatment	Element	1	2	3	4	5	6	7	Avg.*
1	CONF2-A	Feed	5.9	10.8	6.9	6.6	6.3	8.7	4.9	7.1^{a}
		Shelter	4.2	3.5	6.6	0.7	5.6	4.2	10.1	5.0^{a}
		Water	13.2	10.4	3.8	5.6	4.5	6.9	6.6	7.3^{a}
	CONF1-B	Feed	25.7	16.0	14.6	11.1	15.3	12.8	10.8	15.2^{a}
		Shelter	4.5	5.9	7.3	5.2	4.5	5.2	1.0	4.8^{b}
		Water	15.3	7.6	6.9	1.0	15.6	16.3	13.9	11.0^{a}
	CONF3-B	Feed	3.5	9.7	6.6	2.4	3.1	2.8	1.7	4.3^{b}
		Shelter	3.8	7.3	18.8	1.7	19.4	11.5	11.8	10.6^{a}
		Water	7.6	3.8	9.0	5.6	11.1	13.5	9.4	8.6^{a}
2	CONF1-A	Feed	9.0	18.4	13.5	10.4	14.2	11.8	18.4	13.7^{b}
		Shelter	15.3	10.8	13.9	16.7	13.5	15.6	22.9	15.5^{b}
		Water	39.2	28.8	25.0	21.2	19.1	22.9	27.1	26.2^{a}
	CONF3-A	Feed	28.8	26.0	16.0	20.3	25.3	19.8	14.9	21.6^{a}
		Shelter	17.7	3.5	9.0	6.9	3.8	4.9	6.3	7.4^{b}
		Water	0.3	1.0	7.3	3.5	3.1	6.3	5.9	3.9^{b}
	CONF2-B	Feed	45.8	40.3	27.8	32.6	40.3	19.1	22.2	32.6^{a}
		Shelter	8.3	5.9	6.3	6.9	7.6	5.9	7.3	6.9^{b}
		Water	3.5	1.0	6.9	5.6	4.9	3.8	6.3	4.6^{b}
3	CONF2-A	Feed	9.4	10.1	7.6	2.4	11.8	21.2	2.8	9.3^{b}
		Shelter	16.3	21.9	11.5	14.2	16.0	16.3	17.7	16.3^{a}
		Water	4.9	2.4	5.2	2.8	1.0	3.1	1.7	3.0^{c}
	CONF1-B	Feed	13.5	8.3	13.2	5.6	12.5	8.3	1.4	9.0^{b}
		Shelter	13.9	8.0	3.8	3.8	11.1	3.5	7.3	7.3^{b}
		Water	27.4	20.8	16.0	22.9	16.7	13.9	21.5	19.9^{a}
	CONF3-B	Feed	15.6	6.6	8.0	11.1	13.5	8.0	10.1	10.4^{a}
		Shelter	7.6	9.4	2.4	3.8	7.3	5.6	5.2	5.9^{b}
		Water	6.9	7.6	7.6	4.5	5.2	5.6	4.9	6.1^{ab}
4	CONF1-A	Feed	18.1	21.2	26.4	14.9	17.4	16.7	11.8	18.1^{a}
		Shelter	18.4	15.6	14.2	14.6	14.0	12.4	10.4	14.2^{b}
		Water	21.2	13.9	11.5	18.8	14.6	12.5	13.2	15.1^{ab}
	CONF3-A	Feed	17.0	9.0	21.2	21.5	21.5	22.2	31.6	20.6^{a}
		Shelter	16.3	14.6	8.3	9.7	12.8	4.9	3.1	10.0^{b}
		Water	9.7	8.7	3.1	7.6	5.9	6.6	3.5	6.4^{b}
	CONF2-B	Feed	17.4	13.5	9.4	10.1	15.3	14.9	22.6	14.7^{a}
		Shelter	11.8	9.7	4.9	6.9	8.3	13.2	10.4	9.3^{b}
		Water	11.1	21.5	14.9	16.7	9.4	9.4	8.0	13.0^{ab}

*Standard error of all LSM was 1.6.

[abc]Average percentages within CONF of respective Period not connected by the same letter are significantly different.

Table 2.3 Percent of time horses spent grazing by Period within pasture plots of repeated CONF, in which Periods 1 and 3 had identical CONF as did Periods 2 and 4. Data are presented as LSM.

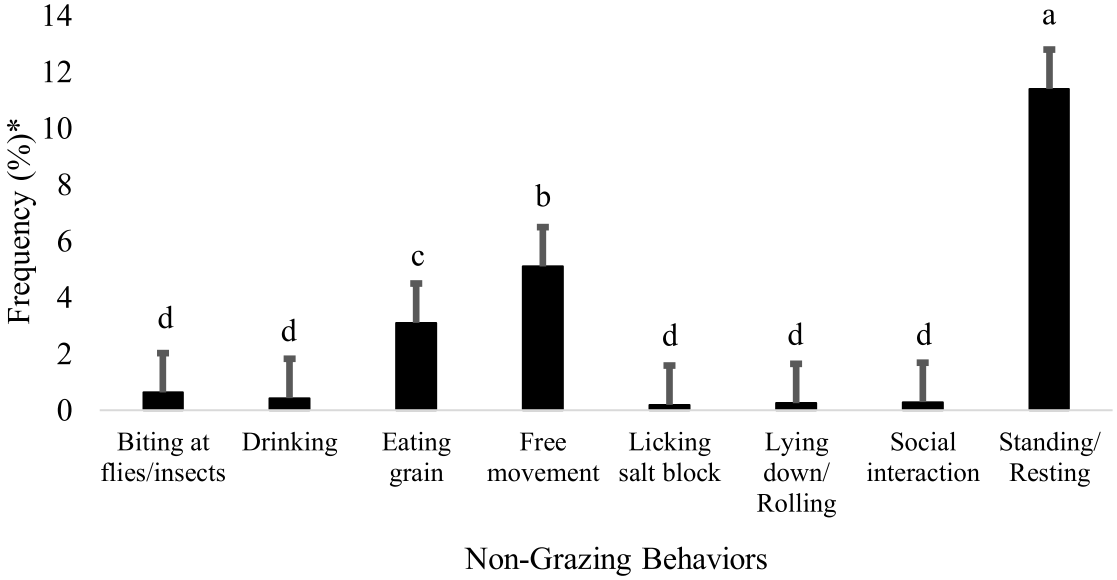
		Day							
Period	Treatment	1	2	3	4	5	6	7	Avg.*
1	CONF2-A	64.0	77.3	89.3	86.7	82.0	78.7	80.0	79.7[a]
	CONF1-B	74.7	44.0	80.7	82.7	80.0	71.3	66.0	71.3[a]
	CONF3-B	71.3	69.3	76.0	72.7	70.0	69.3	73.0	71.7[a]
3	CONF2-A	81.5	75.3	87.3	86.0	71.3	88.7	61.9	78.9[a]
	CONF1-B	78.5	78.7	70.7	86.0	75.3	68.0	85.9	77.6[b]
	CONF3-B	82.2	89.9	83.8	89.3	67.3	80.0	58.9	78.8[b]
2	CONF1-A	83.3	77.3	72.7	92.0	78.7	82.7	76.0	80.4[a]
	CONF3-A	85.3	66.0	73.3	72.7	77.3	68.7	53.0	70.9[a]
	CONF2-B	81.3	67.3	65.3	64.7	79.3	76.7	74.0	72.7[a]
4	CONF1-A	75.5	65.3	82.7	75.3	79.3	79.3	59.9	73.9[b]
	CONF3-A	83.0	77.3	78.7	84.7	93.3	80.0	95.9	84.7[b]
	CONF2-B	77.0	86.7	86.0	84.7	82.7	79.9	77.5	82.1[b]

*Standard error of all LSM was 1.4.

[ab]Average percentages within repeated CONF of respective Periods not connected by the same letter are significantly different.

The second most occurring behavior was Standing/Resting followed by Free Movement and Eating Grain (11.4%, 5.1%, and 3.1% ± 1.4, respectively; Fig. 2.5). The remaining non-grazing activities (Drinking, Social Interaction, Biting at Flies/Insects, Lying down/Rolling, and Licking Salt Block) occurred less than 0.7% of the time observed. There were no differences in these behaviors across the three observation times, as well as no correlation of behaviors within plots of identical configuration.

Figure 2.5 Frequency of horses performing non-grazing behaviors across observation times. Data are presented as LSM with SEM error bars.



*Standard error of all LSM was 1.4.

^{abcd}Behaviors not connected by the same letter are significantly different ($P < 0.05$)

Discussion

Equine location within pasture – GPS

Location in respect to the elements varied within each Period as well as CONF in which horses spent the most time within proximity of the feed element. Configuration 3-A contained the second and third highest counts of horses within 23 m of the concentrate feeding area. Random changes in forage composition and quality were also recorded within CONF3-A. The forage changes may impact grazing or non-grazing activities and thus influence the time spent around the feeding location. However, to the authors' knowledge, the effect forage composition and quality have on equine behavior around required elements is minimal and should be further investigated. Due to the lack of literature regarding the effect required elements have on the grazing distribution of horses, appropriate comparisons to the current trial were made using previous findings in cattle. A study evaluating the grazing distribution of cattle with dehydrated molasses supplement blocks observed a greater forage utilization of cattle across pastures with the dietary supplement than those without (Bailey and Welling, 1999). Forage utilization and stubble height measurements showed cattle grazed more heavily within 20 to 200 m from the dietary supplement than in corresponding control areas (Bailey and Welling, 1999). McDougald and colleagues (1989) investigated the use of a dietary supplement, to manipulate cattle grazing location into less productive pasture areas. They determined, by moving supplemental feeding location away from water sources and into underutilized areas, the impact of cattle on residual dry matter in riparian pasture areas was greatly reduced from 48 to 1% over a three-year period. The current study did not determine use of feeding location in less desirable pasture areas or impact on plant or soil health, yet movement of supplemental feeding into such areas provides opportunity for future research.

Ares (1953) found similar results by distributing the grazing efforts of cattle through the placement of a meal-salt ration and compared this in relation to positioning of water. This study found an 84% increase in use of pasture when the meal-salt was located away from the water as opposed to next to it and determined this positioning of the feed supplement resulted in the most efficient grazing of range forage. This preference for spending time near concentrate feeding area was also observed in the current study; however, placement of feed near water was not investigated as each element was a consistent 56 m apart. The lesser influence of water in comparison to feed on P23 in the current study, however, did conflict with Ganskopp (2001) who found the movement of water to be the most effective tool for altering cattle distribution where a dietary supplement, salt, had less of an impact. It should be noted that pastures evaluated in this cattle study were much larger than those in the current study, 800 ha versus < 1 ha, respectively. The location of elements in the much larger area may have adverse effects on grazing distribution than when confined to much smaller areas. Additionally, differences between the previous studies and current could be due to the preferences of the dietary supplement types by cattle compared to horses as well as the time of year, and lack of shelter.

The use of the man-made shelters was minimal in the current study yet horses were not timid of the structures and were occasionally visually observed grazing under and around them. Heleski and Murtazashvili (2010) discussed that type of artificial shelter in addition to its isolation, ventilation, and orientation could affect the horses' decision to use. Snoeks and colleagues (2015) found domestic horses used shelter approximately half of the observed time, with increased values seen in study determined cold and hot temperatures. A potential reason for the conflicting use of shelters with the current study could be due to the average temperature not exceeding the horses' thermal neutral zone of 25°C (Morgan, 1998). Holcomb and colleagues (2014) determined that individually housed horses preferred foraging in shaded areas. That study was conducted on dry-lots in which forage was provided under open-sided shade structures, indicating there was likely limited forage, which was not the case in the current study. Despite the lack of shelter use observed in the current study, providing shade is still warranted to ensure best management practices, especially in extreme weather conditions as can be observed in the Southeastern United States.

Behavior

It should be noted that horses did tend to visually remain in eyesight of pairs within other pasture plots. However, no matter the configuration that elements were placed within pasture plots, horses spent more time grazing in comparison to other activities. Grazing was expected to be the most frequently occurring behavior, as horses graze between 14 to 17 hours a day (Duncan, 1992; Fleurance et al., 2006; Edouard et al., 2009). Snoeks and colleagues (2015) determined grazing to be the most observed behavior, with 'standing' closely following as in the current study. In natural conditions, Preswalski's horses grazed, rested and moved more than 90% of the time observed, as also comparable to the current study where horses completed the same behaviors in a pasture environment for just over 90% of their daily allowance (van Dierendonck et al., 1996).

Conclusion

Pasture access provides horses with an efficient source of nutrients as well as offers numerous health benefits. However, horses possess the unique behavior to graze forages at an intense level, causing potential damage to plant health and environmental risks. Moving feeding location frequently may alter horse location within a pasture, thus distributing animal concentration accordingly and decreasing the risk of overgrazing. This method may provide equine owners with an alternative or serve as a complement to previously existing pasture management techniques. Further research is warranted to determine the effects that required elements have in varying seasons and forage availability.

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CHAPTER THREE

MANIPULATION OF FEEDING FREQUENCY AND MECHANISM TO DISTRIBUTE LOCATION AND BEHAVIOR OF HORSES IN A PASTURE ENVIIRONMENT

Abstract

Horses are influenced by supplementary concentrate feed and have shown to prefer it over other feedstuffs such as forage. The objectives of this study were to determine the effects that frequency of feedings and feeding mechanism have on grazing distribution and behavior of horses. Eight mature mares were paired and assigned to one of four individual pasture plots (1.8 ha each) for four 7d periods with a 72hr washout between each period totaling 40d. The combination of two feeding frequencies (2x/d or 4x/d) and two feeding mechanisms [manual (M); automatic (A)] made up four treatments (2 and 4x/d automatic; 2 and 4x/d manual; 2A, 4A, 2M and 4M, respectively) that were implemented in a Latin square design with repeated measures across pasture plots. Depending on assigned treatment, horses were fed a hay balancer to manufacturer's recommendation (0.45kg or 0.23kg; 2 or 4x/day, respectively; Nutrena® Empower® Topline Balancer, Cargill Incorporated©, Minneapolis, MN) at 0715 and 1715 or 0715, 1015, 1315 and 1715 daily with *ad libitum* access to portable water tanks. Horses were fitted with global positioning systems (GPS; Trak-4 GPS Tracker, Pryor, OK), logging measurements every 10min. Horses were also visually observed for four 2hr periods (MOR; NOON; MID; EVE) during the 7-d grazing periods, with behaviors recorded every 5 min via scan-sampling. Trained observers classified horse behavior as either grazing or other activity. Linear mixed models were developed that related occurrence of

behaviors or horse presence within 23 m (P23) of feed (F), shelter (S) and water (W) to fixed effects of element and behavior; the random effects of treatment, period, activity, time, day and horse; and interactions. An ANOVA was used to determine if the fixed effects were significant, followed by Fisher's Protected LSD to compare means. Treatment had an effect on P23 of elements in that horses spent more time ($P < 0.05$) in relation to F in 2A compared to 2M, 4M, and 4A (11%, 9.8%, 9.5%, and 9.2% ± 1.2, respectively). Horses were seen grazing more than ($P < 0.05$) other observed behaviors and more often in 2M compared to 2A, 4A, and 4M ($P < 0.01$; 80.4%, 74.8%, 74.9% and 72.6% ± 0.5). Thus, altering feeding frequency and mechanism may impact horse location within a pasture environment and grazing behavior. Further research is warranted to confirm increased feeding frequency via an automatic mechanism as an effective equine pasture management strategy.

Introduction

Horses express a drive to consume supplemental feedstuffs, such as concentrate, whether fed alone or in conjunction with forage. Henneke and Callahan (2009) reported high intake of *ad libitum* concentrate when horses were simultaneously offered *ad libitum* access to hay. Traditional supplemental feeding practices largely revolve around ease of management and thus, horses are commonly fed twice a day (Harris, 1999). However, previous research has also explored the effects that multiple daily feedings have on horse health and behavior (Clarke et al., 1990; Cooper et al., 2005). Horses are naturally trickle feeders, thus, dividing supplemental feed rations into smaller more frequent meals may satisfy horse's gastrointestinal tract and decrease unwanted feeding behaviors. Increasing the frequency of concentrate meals allows horses more time to consume meals and decrease oral stereotypies (Cooper et al., 2005).

Offering horses multiple meals daily may result in added labor for owners, leading to the investigation of automatic feeders as a feeding management technique. Horses adapt to the automatic concentrate feeding systems (CFS) quickly and the use of small frequent meals further enhance horse movement and lying down behaviors as opposed to within traditional individual stalls (Hoffman et al., 2012). Frölich and colleagues (2004) evaluated a similar management system and determined the automatic feeders to efficiently dispense several meals throughout the day in a controlled manner as well as reduced food rivalry and increased activity in a group setting.

The manipulation of feedstuffs has been previously shown in the literature to distribute the location of grazing animals in a pasture environment. The movement of dehydrated molasses supplement lured cattle to improve uniformity of grazing underutilized rangeland (Bailey and Welling, 1999). Perron et al. (2022) also investigated the movement of required maintenance elements and found that concentrate feed was most influential in altering where horses allocated their time, compared to shelter or water. However, to the authors' knowledge, frequency of meals and use of automatic feeders have not been assessed within an equine pasture environment, which suggests further investigation should be considered to confirm this a management technique in horses. The primary goals of this research were to determine the effects that frequency of feedings and feeding mechanism have on grazing distribution and behavior of horses.

Materials and Methods

This research was approved by the Institutional Animal Care and Use Committee of Clemson University (IACUC Protocol #: 2020-037).

Animals and Environment

The trial was conducted at the Clemson University Equine Center in Pendleton, SC. The trial lasted for 40 days, beginning the 8th of October and ending November 16th of 2021. Horses grazed four pasture plots approximately 1.8 ha in size that were mowed to a sward height of approximately 20 cm prior to grazing. Eight mature mares (7 American Quarter Horse and 1 Warmblood; 13.8 ± 1.3 yrs, 500 ± 13.2 kg) underwent grazing at a stocking rate of 0.9 ha/horse (Bott et al., 2013). The pasture stand had not been renovated in over ten years prior to the current trial including no fertilization or seeding, and thus forages are of native composition common to Zone 8. The soil in all pasture plots consisted of Cecil sandy loam with approximately 80% at a slope of 2-6%

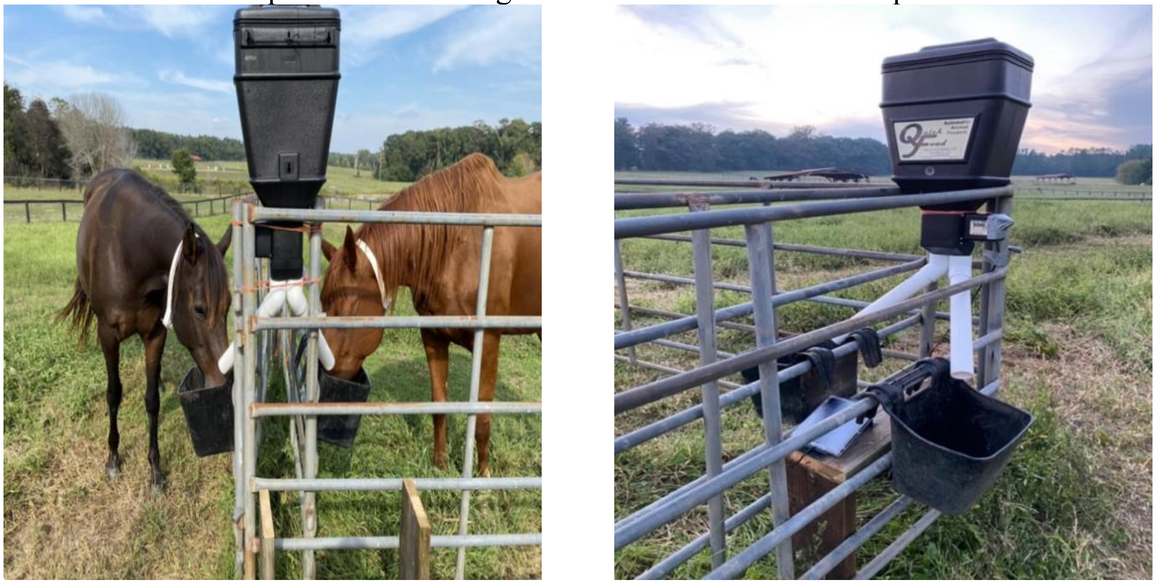
and the remaining at 6-10%. Horses had the majority of a free line of sight to horses grazing in other pasture plots with less than 10% of Plot 3 not visible by the remaining plots. Climate measurements were also acquired from the National Weather Service throughout the course of the trial, with an average temperature of 14.2°C ± 0.76, range of -2.22 to 28.3°C, and average precipitation of 1.85 mm ± 0.87.

Experimental Design

Horses were randomly paired to graze four treatments, which were a combination of two feeding frequencies (2x/d or 4x/d) and two feeding mechanisms [manual (M); automatic (A); 2 and 4x/d automatic; 2 and 4x/d manual; 2A, 4A, 2M and 4M, respectively]. Plots were 1.8 ha each and defined using electric 38 mm polytape (Pasture Management Systems®, Inc., Mt. Pleasant, NC). All pasture plots were randomly assigned a treatment and grazed simultaneously, each by a different pair of horses within one 7-d period. The trial consisted of four 7-d periods, subsequently referred to as Periods 1-4, and four preceding 72-hr washout phases. Each period was initiated with a 72-hr washout in which horses were placed in individual outdoor stalls with no pasture access. During washout periods, horses were fed *ad libitum* long-stem forage along with a concentrate hay balancer fed to manufacturer's recommendation twice daily at 0715 and 1615 (0.23 kg of Nutrena® Empower® Topline Balancer, Cargill Incorporated©, Minneapolis, MN). Depending on the assigned treatment while in the pasture plots, horses were also fed concentrate hay balancer two (0.45 kg; at 0715 and 1615) or four (0.23 kg; at 0715, 1015, 1315 and 1715) times daily. For all treatments, one feed bucket was hung on either side of a 3.05 m panel to minimize competition for the balancer.

During manual feeding, volunteers placed feed directly into buckets, while automatic feeders (Quickfeed©, Sanford, FL, USA) were placed in between panels and dispensed pre-determined amount of feed into buckets (Fig. 3.1). Prior to each period, the appropriate automatic feeders were filled with the amount of feed needed for that specific 7-d period. Within each pasture plot, supplemental feeding area (F), shelter (S) and water (W) were located an equal 56 m apart as demonstrated in previous literature [8]. Shelters were portable man-made structures with canvas tops. Horses were given a 1-wk adjustment period to both automatic feeders and shelters pre-trial. During the adjustment period, feed (0.45 kg) was dispensed twice daily at 0715 and 1615. Water was provided *ad libitum* in portable 100-gallon stock tanks.

Figure 3.1 Placement of automatic feeders (Quickfeed©, Sanford, FL, USA) and hanging buckets on a 3.05 m panel also dividing horses to minimize food competition.



Pasture Sampling and Analysis

Prior to the start of each period, forage composition and quality were determined through collection of ten samples from each pasture plot. Pasture composition was visually assessed using the double DAFOR scale, a measurement technique to assess the relative abundance of forage and weed species within a 0.5-m^2 quadrat (Abaye et al., 1997; Virostek et al., 2015). Forage species that covered >75% of the area assessed were assigned “dominant” (D); “abundant” (A) to species that covered 50-75%; “frequent” (F) to species that covered <50% but were well distributed in the area; “occasional” (O) species were those found a few times; and “rare” (R) are species that only occurred one or two times in the given area. Post-composition analysis, forage within the 0.5-m^2 quadrat were collected via hand-clippings to ground level and subsequently dried at 55°C for 48 hr in a forced-air oven (Weinert and Williams, 2018). Dry samples were ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Ground samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) content. Neutral detergent fiber and ADF concentrations were determined using an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NW) and corrected for ash concentration. Sodium sulfite and α-amylase (Sigma no. A3306: Sigma Chemical CO., St. Louis, MO) according to Van Soest and colleagues (1991), were included for NDF analysis.

Behavior Sampling

During the grazing periods, horses were fitted with a Global Positioning System (GPS) unit (Trak-4 GPS Tracker, Pryor, OK) mounted onto individual identification collars (Martinson et al., 2017). As suggested by previous literature, horses carried collars for a 1-week adjustment period prior to the study (Ganskopp, 2001). GPS units remained mounted on the upper neck of horses for all 7-d grazing periods, logging location measurements every 10 min, thus producing an expected 4128 recorded positions per horse. The GPS response variable included frequency of horses present within 23 m in relation to elements. The 23-m distance was utilized due to being the halfway point between elements.

Horses were also live observed for four, 2-hour periods [0700-0900 (MOR); 1000-1200 (NOON); 1300-1500 (MID); 1600-1800 (EVE)] per day throughout all 7 d of each period. Horses were conditioned to their designated pasture plot 12 hr before the first observation of each period began. Activity was recorded using the scan sampling method (Altman, 1974), where a 5-s scan of the horses was made every 5 min and the activity of each individual was recorded. Two individuals from the same set of observers throughout the trial were randomly assigned to each three, 2-hr timepoints to both observe and concur each horse's behavior within all pasture plots. Horse behavior was classified as either Grazing (actively consuming pasture forage) or non-grazing activity, otherwise recorded as Free Movement, Drinking, Standing/Resting, Social Interaction, Biting at Flies/Insects, Lying Down/Rolling, Eating Grain, Licking Salt Block, or Disrupting Feeder.

Statistical Analyses

Two linear models were developed that related forage composition to the fixed effects of plots and period; interactions and forage quality to fixed effects of plots and period; and interactions. A linear mixed model was established that related horse presence within 23 m to the fixed effect of element; the random effect of treatment, horse, day and period; and interactions. Linear contrasts were used to partition the treatment effect of the component due to feeding frequency and mechanism. A second linear mixed model then related the frequency of activity to the fixed effect of behavior; the random effects of treatment, horse, time, day and period; and interactions. Linear contrasts were used to partition the treatment effect of the component due to feeding frequency and mechanism.

An ANOVA was used to determine if the fixed effects were statistically different. If the fixed effects were found to be significantly different at alpha = 0.05, then Fisher's Protected Least Significant Difference was used to compare the means. All statistical analyses were completed using JMP version 15 (2019 SAS Institute Inc.). Data were presented as LSM ± SEM. Examination of residuals plots combined with tests (Shapiro-Wilk and Levene) were used to assess ANOVA assumptions concerning normality and stable variance. ANOVA independence assumptions were addressed by including all possible factors (that could lead to clustering and correlation of observations) in the linear mixed models.

Results

Pasture

Within all four pasture plots, composition consisted of three forage species including Broad leaf weed, Tall Fescue (*Schedonorus pheonix*), White Clover (*Trifolium repens*), and dead material or bare ground categorized as 'Other'. All forage species were found during each period and pasture plot (Table 3.1). Overall, Fescue was the most occurring species across all plots and periods ($P < 0.05$; 2.9 ± 0.13). No differences were observed between Broad leaf weed and White Clover, yet the two were greater than

‘Other’ (2.1, 1.9 and 1.3 ± 0.13, respectively). Differences in species presence were also observed across periods within pasture plots.

Table 3.1 Comparison of forage species by period within plots of varying treatments [Period 1 = 2A, 2M, 4A and 4M; Period 2 = 2M, 4A, 4M and 2A; Period 3 = 4A, 4M, 2A and 2M; Period 4 = 4M, 2A, 2M and 4A; for plots 1-4 , respectively]. The double DAFOR scale was used to make accurate comparisons [Dominant (D)=5, Abundant (A)= 4, Frequent (F)=3, Occasional (O)=2, and Rare (R)=1. Data are presented as percent LSM ± SEM.

Plot	Period	Treatment	Forage*				
			Bermudagrass	Broad leaf weed	Tall Fescue	White Clover	Other
1	1	2A	3.3^{a}	1.9^{b}	2.0^{c}	0.7^{b}	3.1^{a}
	2	2M	3.1^{a}	1.9^{b}	3.3^{a}	2.3^{a}	0.3^{c}
	3	4A	2.6^{b}	2.1^{a}	2.0^{c}	0.4^{b}	3.3^{a}
	4	4M	3.2^{a}	2.3^{a}	2.5^{b}	0.7^{b}	2.5^{b}
2	1	2M	3.1^{a}	2.0	3.9^{a}	2.3^{a}	1.3^{b}
	2	4A	2.8^{a}	2.1	3.3^{a}	0.4^{c}	3.0^{a}
	3	4M	2.8^{a}	2.1	3.5^{a}	2.5^{a}	1.3^{b}
	4	2A	3.0^{a}	1.0	3.7^{a}	1.2^{b}	1.6^{b}
3	1	4A	2.7^{ab}	2.3	1.0^{b}	1.2^{a}	3.0^{a}
	2	4M	3.1^{a}	2.2	3.5^{a}	1.0^{a}	2.2^{b}
	3	2A	2.2^{b}	1.2	3.8^{a}	1.4^{a}	1.8^{b}
	4	2M	1.8^{b}	1.4	3.9^{a}	0.9^{a}	1.5^{b}
4	1	4M	2.0^{b}	1.9	2.2^{b}	1.6^{b}	2.5^{a}
	2	2A	2.6^{a}	2.9	1.7^{b}	2.3^{a}	2.4^{a}
	3	2M	2.2^{ab}	0.9	4.6^{a}	0.8^{c}	1.8^{b}
	4	4A	2.4^{a}	2.9	1.2^{b}	0.9^{c}	2.9^{a}

*Standard error of all LSM were 0.5.

^{abc}Identical forage species across periods within plots not connected by the same letter are significantly different ($P < 0.05$).

Mean ADF values varied among forages across periods within pasture plots (Table 3.2). Within Plot 1, ADF values tended to decrease after period 1 whereas the remaining plots fluctuated across periods ($P < 0.05$). Mean NDF values were consistent over the course of the trial.

Table 3.2 Nutritive values of forage available by period within plots of varying treatments [Period 1 = 2A, 2M, 4A and 4M; Period 2 = 2M, 4A, 4M and 2A; Period 3 = 4A, 4M, 2A and 2M; Period 4 = 4M, 2A, 2M and 4A; for plots 1-4, respectively]. Data presented as percent LSM ± SEM.

Plot	Period	Treatment	Quality (%)*	
			NDF	ADF
1	1	2A	66.9^{a}	44.6^{a}
	2	2M	62.6^{a}	41.4^{b}
	3	4A	61.4^{a}	43.6^{ab}
	4	4M	63.8^{a}	41.6^{b}
2	1	2M	59.6^{a}	38.9^{c}
	2	4A	63.4^{a}	41.2^{bc}
	3	4M	60.1^{a}	43.7^{a}
	4	2A	64.4^{a}	42.2^{ab}
3	1	4A	57.2^{a}	36.6^{c}
	2	4M	60.7^{a}	36.8^{c}
	3	2A	62.9^{a}	40.8^{ab}
	4	2M	60.5^{a}	38.4^{bc}
4	1	4M	58.8^{a}	35.8^{bc}
	2	2A	49.2^{a}	34^{c}
	3	2M	56.4^{a}	42.5^{a}
	4	4A	56.6^{a}	35.7^{bc}

*Standard error of all LSM were 6.6 and 0.86, respectively.

[abc]Percentages with differing letters within Plot rows are significantly different ($P < 0.05$).

Pasture Location via GPS

Over the 28 days that treatments were in effect, each GPS unit was expected to record 144 positions daily and 4,032 total. The Trak-4 GPS units contained hardware and logical processing to calculate position based on GPS satellite signals, tracking location by user-selected time interval and movement of unit, potentially producing more or less than the expected number of positions. Six of the eight units recorded 90.2% to over

100% of the expected number of positions, delivering an average of 4060 ± 162.8. The remaining two units experienced technical difficulties within one of the four periods and thus only reported 55.8 and 66.6% of the expected positions throughout the trial. Therefore, results were calculated based on the daily expected number of positions.

Over the total GPS positions recorded throughout each pasture plot, location within 23m of an element, regardless of element type, ranged from 6.3 to 31% between horses. Regardless of feeding frequency or mechanism, horses frequented F most often compared to W and S (*P* < 0.0001, Table 3.3).

Table 3.3. Main effect of element in horse P23 of an element, regardless of period, day, or treatment, across 4, 7-day periods by feeding frequency and mechanism. Data are presented as percent LSM ± SEM.

Element	LSM (%)	SEM
F	9.9^{a}	1.2
S	7.0^{b}	1.2
W	4.9^{c}	1.2

^{ab}LSM not connected by the same letter are significantly different (*P* < 0.0001).

Feed was further evaluated as it was the most influential element in horse location within 23 m of the elements. Across F only, the significant main effect was period. Period 1 displayed the lowest occurrence (*P* < 0.05; 8.3% ± 1.7) of P23 of F while Periods 2, 3 and 4 were not statistically different (10.2%, 10.3%, 10.9% ± 1.7, respectively) from each other. No differences for P23 of F across day or treatment were observed; however, a significant interaction (*P* = 0.003) between period and treatment was noted and can be further explained in Table 3.4.

Table 3.4. Main effect of the interaction of period and treatment with location of horse in pasture within 23 m of F. Data are presented as percent LSM ± SEM.

Period	Treatment	LSM(%)	SEM
1	2A	10.5[a]	2.7
	2M	8.2[a]	3.4
	4A	4.4[b]	2.7
	4M	9.9[a]	3.4
2	2A	9.1[b]	3.4
	2M	8.8[b]	2.7
	4A	15.2[a]	3.4
	4M	7.8[b]	2.7
3	2A	9.7[a]	2.5
	2M	12.4[a]	3.4
	4A	5.9[b]	3.1
	4M	13.2[a]	3.4
4	2A	15.4[a]	3.4
	2M	9.9[a]	2.7
	4A	11.0[a]	3.4
	4M	7.2[b]	2.7

[ab]LSM within Period not connected by the same letter are significantly different ($P <$ 0.003)

There was also a period effect on P23 of an element, regardless of element type (P = 0.00012). Horses were located within 23 m of an element, regardless of element type, in Period 1, more so than Periods 2 through 4 (Table 3.5).

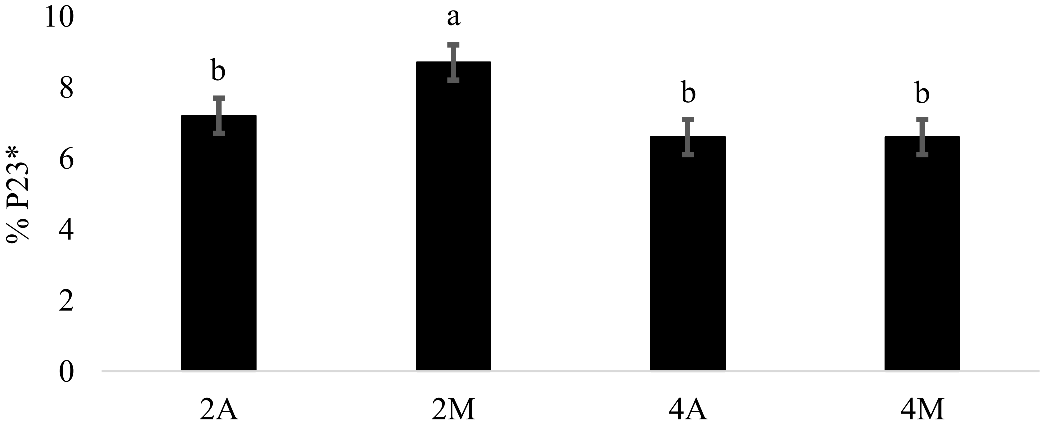
Table 3.5. Main effect of period across feeding frequency and mechanism on location of horse in pasture within 23 m of feed, shelter, or water. Data are presented as percent LSM ± SEM.

Period	LSM (%)	SEM
1	8.5[a]	1.2
2	6.4[b]	1.2
3	7.0[b]	1.2
4	7.2[b]	1.2

[ab]LSM not connected by the same letter are significantly different ($P < 0.0001$).

When accounting for both frequency and mechanism treatments, there was an effect on P23 of an element, regardless of element type, in which horses were near 2M more often (Figure 3.2; P < 0.001; 8.7%) than 2A, 4A and 4M (7.2%, 6.6% and 6.5%, respectively).

Figure 3.2. Frequency of horse presence within 23m (P23) of an element, regardless of element type, within feeding frequency (2x/d vs 4x/d) and mechanism (automatic vs manual), across 4, 7-d periods. Data are presented as LSM with SEM error bars.



*Standard error of all LSM was 1.2.

^{ab}Element within treatment not connected by the same letter are significantly different (*P* < 0.001)

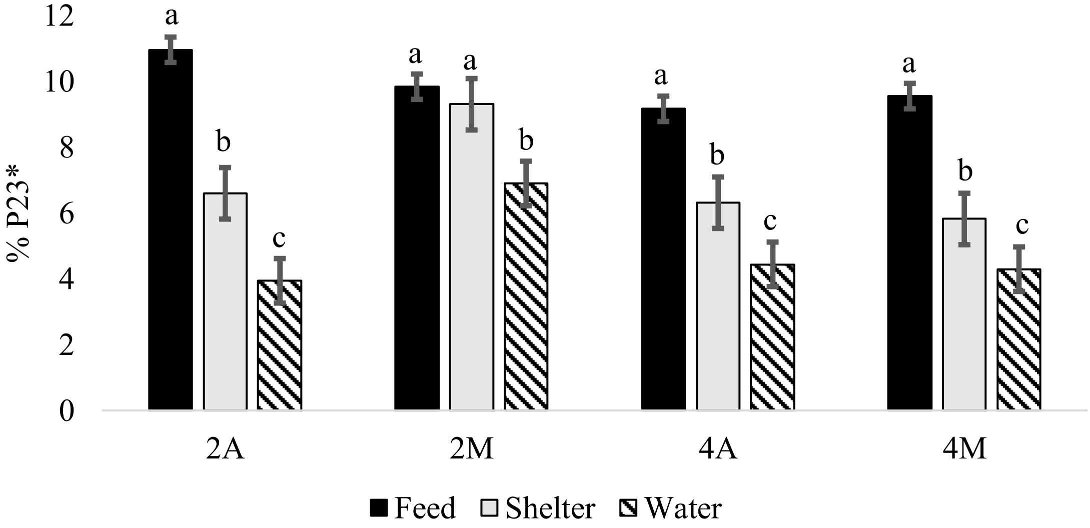
Further comparison of feeding frequency on P23 of an element, regardless of element type, determined horses spent more time in relation to the 2x/d feeding frequency more so than the 4x/d (*P* < 0.0001). Comparison of feeding mechanism on P23 of an element, regardless of element type, was also statistically significant, in that horses spent more time near the manual feeding mechanism compared to the automatic (*P* = 0.02). Comparisons of feeding frequency and mechanism can be found in Table 3.6.

Table 3.6. Comparison of feeding horses 2 vs. 4x/d either by manual or automatic feeders; and of feeding horses manually vs. automatically by 2 or 4x/d, across 4, 7-d grazing periods with location of horse in pasture within 23 m of feed, shelter, or water. Data are presented as *F*-statistic

	F-statistic	p-Value
Frequency	17.9	*P* < 0.0001
Mechanism	5.2	*P* = 0.02

In addition to the above effects on horse P23, there were equine pasture management-relevant interactions including element by treatment, element by period by treatment and element by period by day by treatment. Treatment by element interaction revealed that horses spent more time (*P* = 0.0013) within P23 of F in 2A compared to 2M, 4M, and 4A, further demonstrated in Figure 3.3.

Figure 3.3. Frequency of horse presence within 23m (P23) of an element within feeding frequency (2x/d vs 4x/d) and mechanism (automatic vs manual), and the 4, 7-d periods. Data are presented as LSM with SEM error bars.



*Standard error of all LSM was 1.2 for 2A, 2M and 4M; 1.3 for 4A.
^{abc}Element within treatment not connected by the same letter are significantly different (*P* = 0.0013).

An additional interaction comparison of feeding frequency by element determined horse P23 of an element, regardless of type, was not influenced by 2 or 4x/d feeding frequency (*P* = 0.44). The interaction comparison of feeding mechanism by element, however, did have an effect on P23 across an element, regardless of element type, in that horses were near F more than the remaining elements while the automatic feeding mechanism was in use ($P < 0.0001$). Interaction comparisons of feeding frequency and mechanism by element can be found in Table 3.7.

Table 3.7. Interaction comparison of feeding horses 2 vs. 4x/d either by manual or automatic feeders by element (mechanism); and of feeding horses manually vs. automatically either by 2 or 4x/d by element (frequency), across 4, 7-d grazing periods with location of horse in pasture within 23 m of feed, shelter, or water. Data are presented as *F*-statistic.

	F-statistic	P-Value
Frequency	0.8	$P = 0.44$
Mechanism	47.4	$P < 0.0001$

Differences in P23 of an element, regardless of element type, were also observed within the interaction of treatment, period and element (Table 3.8). Within Period 1, horses were recorded most near S in 4M ($P < 0.05$; 15.6% ± 1.2). Similarly, in Period 3, horses were observed most near S in 2M ($P < 0.01$; 17.6% ± 2.6). As for Periods 2 and 4, horses were more frequently detected in relation to F ($P < 0.05$), though in different treatments (4A and 4M vs. 2A and 2M, respectively) for each of these periods.

Table 3.8. Main effect interaction of element, period and treatment with location of horse in pasture within 23 m of feed, shelter or water. Data are presented as percent LSM ± SEM.

Period	Treatment	LSM (%)			SEM
		Feed	Shelter	Water	
1	2A	6^{de}	4.4^{e}	1.1^{f}	2.0
	2M	8.3^{cd}	7.9^{cde}	12.8^{ab}	2.5
	4A	8.9^{cd}	9.0^{cd}	12.0^{ab}	2.7
	4M	9.9^{bc}	15.6^{a}	5.6^{de}	3.4
2	2A	9.1^{bc}	10.6^{b}	1.6^{ef}	2.5
	2M	4.3^{de}	6.0^{cd}	1.4^{f}	2.0
	4A	15.2^{a}	1.2^{ef}	1.1^{ef}	2.5
	4M	12.4^{ab}	4.1^{def}	10.2^{b}	2.0
3	2A	11.4^{bdf}	5.0^{eh}	7.2^{cegh}	1.8
	2M	12.4^{bce}	17.6^{a}	6.3^{dfgh}	2.5
	4A	1.0^{fh}	0.7^{h}	0.0^{h}	2.3
	4M	13.2^{abcdg}	1.3^{h}	0.3^{h}	2.5
4	2A	15.4^{ab}	4.5^{d}	3.8^{d}	2.5

2M	14.4^{ab}	5.8^{cd}	7.1^{cd}	2.0
4A	11.0^{bc}	13.7^{b}	4.1^{d}	2.5
4M	2.7^{d}	2.2^{d}	1.1^{d}	2.0

^{abcdefgh}Average percentages within periods not connected by the same letter are significantly different.

Behavior

Observers monitored the behavior of the eight mares for a total of 224 h, resulting in a significant main effect of Grazing activity being the most observed behavior ($P < 0.0001$; Table 3.9). Other observed non-grazing behaviors: Standing/Resting, Free Movement, and Eating Grain were statistically different from each other ($P < 0.0001$), and the remaining non-grazing behaviors were each less than 1% of horses' time. No other main effects showed significant statistical differences; thus, no main effects contrasts were deemed necessary. It should be noted that even though Disrupting Feeder occurred less than 1%, measures were taken to ensure feeders lasted throughout the trial including the assembly of a barrier around feeder lids.

Table 3.9. Main effect of behavior across 4, 7-day periods and behavior observation times of MOR, NOON, MID and EVE. Data are presented as percent LSM ± SEM.

Behavior	LSM (%)	SEM
Grazing	75.7^{a}	0.52
Standing/Resting	15.9^{b}	0.52
Free Movement	3.4^{c}	0.52
Eating Grain	2.6^{d}	0.52
Disrupting Feeder	0.8^{e}	0.52
Drinking	0.6^{ef}	0.52
Lying Down/Rolling	0.3^{ef}	0.52
Biting at Flies/Insects	0.2^{ef}	0.52
Licking Salt Block	0.1^{ef}	0.52
Social Interaction	0.03^{f}	0.52

^{ab}LSM not connected by the same letter are significantly different ($P < 0.0001$).

In addition to main effect of behavior, significant statistical interactions, including behavior by period, behavior by day, behavior by time, behavior by treatment were observed. Period had a statistical effect on frequency of behavior ($P < 0.0001$) with Grazing increasing from Period 1 to 4 and a decrease in Standing/Resting (Table 3.10). Similar to Standing/Resting, Free Movement and Eating Grain statistically decreased from Period 1 to 4. There was no period by behavior differences observed between the remaining behaviors.

Table 3.10. Main effect interaction of behavior and period across 4, 7-day periods behavior observation times of MOR, NOON, MID and EVE. Data are presented as percent LSM ± SEM.

Behavior	Period	LSM (%)*
Grazing	1	67.1^{d}
	2	70.8^{c}
	3	79.0^{b}
	4	85.8^{a}
Standing/Resting	1	21.0^{e}
	2	20.7^{e}
	3	13.6^{g}
	4	8.1^{g}
Free Movement	1	5.1^{h}
	2	3.4^{h}
	3	2.8^{i}
	4	2.8^{ij}
Eating Grain	1	2.7^{ij}
	2	2.5^{ij}
	3	2.3^{ij}
	4	2.2^{ijk}

*Standard error of all LSM were 0.5.

^{abcdefghijk}LSM not connected by the same letter are significantly different ($P < 0.0001$).

As days within periods also progressed (d 1-7), horses were observed to Graze longer, and Stand/Rest less ($P < 0.002$; Table 3.11). Free Movement and Eating Grain, however, were more sporadically distributed across days and showed no statistical significance. As for time of day, Grazing and Free Movement occurred most frequently in the EVE (*P* < 0.01; 82.4% and 25.3% ± 0.52) while Standing/Resting took place more during NOON and Eating Grain in the MOR (25.3% and 4.1% ± 0.52).

Table 3.11. Main effect interaction of behavior and day across 4, 7-day periods and behavior observation times of MOR, NOON, MID and EVE. Data are presented as percent LSM ± SEM.

Behavior	Day	LSM (%)*
Grazing	1	72.4^{c}
	2	74.5^{b}
	3	74.8^{b}
	4	75.5^{b}
	5	75.7^{b}
	6	78.1^{a}
	7	78.9^{a}
Standing/Resting	1	18.4^{d}
	2	16.8^{de}
	3	15.6^{ef}
	4	15.3^{ef}
	5	15.2^{ef}
	6	15.0^{ef}
	7	14.7^{ef}

*Standard error of all LSM were 0.7.

^{abcdefghijk}LSM not connected by the same letter are significantly different (*P* < 0.0001).

The interaction of treatment and behavior was also found statistically significant, where Grazing occurred most often with the highest amount in 2M (*P* < 0.0001; Table 3.12). Other behavior and treatment interaction results replicated that of above, in that frequency of Standing/Resting, Free Movement, and Eating Grain were respectively less than Grazing, and no differences were noted between the remaining behaviors.

Table 3.12. Main effect interaction of behavior and treatment across 4, 7-day periods and observation times of MOR, NOON, MID and EVE. Data are presented as percent LSM ± SEM.

Behavior	Treatment	LSM (%)*
Grazing	2A	74.8^{b}
	2M	80.5^{a}
	4A	74.9^{b}
	4M	72.6^{c}
Standing/Resting	2A	16.0^{e}
	2M	11.7^{f}
	4A	16.6^{e}
	4M	19.1^{d}
Free Movement	2A	3.6^{g}
	2M	3.4^{g}
	4A	3.4^{g}
	4M	3.3^{gh}
Eating Grain	2A	2.2^{ghij}
	2M	2.0^{hij}
	4A	2.9^{ghi}
	4M	3.1^{gh}

*Standard error of all LSM were 0.5.

^{abcdefghij}LSM not connected by the same letter are significantly different ($P < 0.0001$).

Further comparisons between Grazing activity and feeding frequency indicated horses did Graze more often when fed 2x/d vs 4x/d ($P < 0.0001$). Another interaction comparison between Grazing and feeding mechanism was also significant in that horses Grazed more when fed manually vs automatically ($P = 0.002$). Comparisons of feeding frequency and mechanism by Grazing can be found in Table 3.13.

Table 3.13. Interaction comparison of feeding horses 2 vs. 4x/d either by manual or automatic feeders by Grazing; and of feeding horses manually vs. automatically either by 2 or 4x/d by Grazing, across 4, 7-d grazing periods and behavior observations of MOR, NOON, MID and EVE. Data are presented as *F*-statistic

	F-statistic	P-Value
Frequency	55.6	$P < 0.0001$
Mechanism	9.8	$P < 0.002$

Discussion

Equine location within pasture – GPS

In effort to localize distribution of and reduce grazing risks associated with horses at pasture through the use of required maintenance elements, both feeding frequency and mechanism were investigated as pasture management techniques. To the authors' knowledge, only one previous study has explored the effect that maintenance requirement elements have on horse distribution in a pasture environment (Perron et al., 2022). That study found similar results to the current trial, in that horses were found within 23 m of the feeding element more often than shelter and water (Perron et al., 2022). However, element location was not altered in the present study, as it was in the aforementioned research, yet feeding frequency and mechanism were.

Previous literature led the authors to consider feeding frequency and mechanism as a viable pasture management technique (Henneke and Callahan, 2009; Cooper et al., 2005). A horse's motivation to consume supplemental feedstuffs alone or in addition to forage as noted by Henneke and Callahan (2009), drove the ideation of increasing feeding frequency. In addition, while in a stalled environment, increased feeding frequency led to slower concentrate meal consumption and more time spent in relation to the feeding area (Cooper et al., 2005). Although horses had limited access to pasture in that study, they were given a consistent amount of haylage, simulating similar effects of the forage availability in pasture of the current study. However, the current study, resulted in horses allocating their time more near elements during the industry standard 2x/d feeding frequency as opposed to the increased frequency of 4x/d as well as near manual feeders over automatic. Thus, horses were located within 23 m of an element, despite type, most often when manually fed twice a day, though inconsistencies were apparent on a daily basis. It is likely horses needed more time to adjust to the treatments in order to see uniformity of horse location within a pasture due to feeding frequency and/or mechanism. Data loss of GPS positions in Period 3 due to technical difficulties may also have impacted distribution due to treatment consistencies.

When feeding element was evaluated individually, however, horses tended to spend more time near the automatic feeder at both levels of feeding frequency. As the trial progressed, horses were also observed to increase their time spent in relation to the feeding element, indicating an added level of comfort the longer treatments were in effect. A follow-up study design may include longer treatment periods to observe further effects that feeding frequency and mechanism have on horse distribution within a pasture environment.

Future research may also examine varying distance between elements to further determine where horses allocate their time in relation to feed, shelter and water depending on feeding frequency and mechanism. Hoffman and colleagues (2012) recommended the distance between elements, such as feed and water, be as long as possible to motivate horses to distribute their movement. The previous was conducted in a dry-lot, group-housing system and may have adverse effects when applied to a pasture environment in addition to the implementation of feeding frequency and mechanism. Additionally, the purpose of our study was not to study movement, but rather where horses spent their time and what behaviors they completed in those locations.

Behavior

As previous literature (Fleurance et al., 2001; Menard et al., 2002; Lopez et al., 2019) has determined, horses in the current trial spent over half of their daily time grazing, followed by standing and/or resting. Mayes and Duncan (1986) found major feeding times of free-ranging Camargue horses to be after dawn and before dusk, likely due to diurnal patterns and season. The current trial observes similar results in that grazing occurred more often from 1600-1800 compared to the remaining daylight observation hours.

As seen in the GPS results, horses were believed to grow more comfortable within the associated treatments, as grazing frequency increased daily from Period 1 to 4. Furthermore, NDF% tended to decrease as the trial progressed, potentially justifying the increase in grazing frequency as horses have been shown to increase intake as fiber levels decrease (Fleurance et al., 2001). Similar to location distribution, grazing varied by day across all treatments. Again, a longer adaptation period to the treatments or days within trial may have decreased daily inconsistencies.

Overall, occurrence of behaviors was not influenced by feeding frequency nor mechanism; however, a difference was observed specifically on grazing activity. Horses grazed more frequently when fed twice a day versus four times a day, as could be expected since horses reduced their time grazing to consume added meals. Sarker and Holmes (1974) observed similar results as they determined grazing animals that are fed a dietary supplement decrease their grazing time with respect to amount of food supplied and time of day fed.

Horses also grazed more when fed manually versus automatically, potentially denoting horses were more at ease with their traditional feeding method of a physical person delivering food over the sound of the turning auger in the automatic feeder. Although not used in a pasture setting, previous literature investigated the use of concentrate feeding systems in group, dry-lot housed horses and found them to be effective in increasing movement activity as well as reduced food rivalry (Hoffman et al., 2012; Frolich et al., 2004). It should be noted each concentrate feeding system within these previous studies utilized identification sensors to allow all horses equal access to a consistent amount of feed despite hierarchy. The current study did not observe any aggressive behaviors, potentially due to the small quantity of feed provided, partition between feeding stations, and only two horses per pasture (Frolich et al., 2004).

It should be noted that while horses did perform behaviors such as playing with or disrupting of the automatic feeders, no statistical impact was observed. Therefore, the authors feel confident that the increase in feeding frequency did not develop stereotypical behaviors as mentioned in previous studies (Cooper et al., 2005; Nicol, 1999). Even though a minimal (not statistically relevant) amount of this behavior was observed, horses had a cosmetic effect only on automatic feeders, so it is recommended that feeders be completely out of reach of horses to ensure longevity of equipment.

Conclusion

In the current study, horse location and grazing behavior was affected by both feeding frequency and mechanism. The use of manual, twice daily feeding of a concentrated hay balancer at 0.45 kg was most influential in localizing horse activity around required maintenance elements. Future research may increase the time horses have access to treatments in order to see daily consistent location and behavior. Altering feeding frequency and mechanism may impact horse location on a daily basis within a pasture environment and grazing behavior. Further research is warranted to consider increased feeding frequency and automatic mechanism as an effective equine pasture management strategy.

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CHAPTER FOUR

THE INFLUENCE OF SEVERITY OF GASTRIC ULCERATION ON HORSE BEHAVIOR AND HEART RATE VARIABILITY

Abstract

Despite the high prevalence of gastric ulceration in horses, little is known about the behavior and heart rate variability (HRV) indices associated with the severity of this condition. This study examined the effect of severity of gastric ulceration on behavior and HRV indices associated with pain in eight mature University teaching horses, in which ulcers were induced for a coinciding trial. Horses were divided into two groups (n=4) by the severity of gastric ulceration: severe ulcer group [S; scores 3-4], and a mild group [M; scores 0-2]. Horses were housed in 10'x12' stalls and fed 0.5% BW long-stem hay in slow-feeder nets and 0.25% BW concentrate twice daily with *ad libitum* access to water. Dietary modifications were made after endoscopy and horses were administered supportive treatment immediately post-data collection. Horses received a 24h rest period post-endoscopic examination before data sampling began. Behavior and HRV were collected for three consecutive days to account for day-to-day variation and optimize data validity. Behavior was recorded at three 2-h time points per day (Morning, Noon, and Evening) and analyzed in two 15-min increments an hour, totaling nine hours of data per horse. Polar® Equine V800 Heart Rate Monitors were used to record heart rate and HRV, later analyzed using Kubios HRV and MATLAB® software. Mean heart rate and HRV indices, e.g., standard deviation of beat-to-beat intervals (SDRR) and root mean square of successive beat-to-beat differences (RMSSD), the low (LF; sympathetic tone) and high frequency (HF; parasympathetic tone) component of HRV, and their ratio (LF/HF; index

representing the sympatho-vagal balance) were calculated and expressed as response values. The effects of gastric ulcer severity between groups on behavior and HRV were assessed using GLMMs with Tukey's Post hoc test applied to significant results ($\alpha=0.05$) in R (version 3.3.1). Horses with severe ulcers (S) showed higher heart rate (63.3 beats) and LF/HF ratio (5.0%) and reduced SDRR and RMSSD (155.6, 39.73) when compared with M horses (51.08, 3.25, 199.0, 52.57), respectively, (all $P \leq 0.05$). S horses had more frequent eating bouts than M horses (26.6, 12.1 bouts; $P=0.03$). S horses displayed more abdominal kicks, tail switching, tongue activity and pawed more than M horses (5.26, 10.0, 10.35, 6.87 versus 1.32, 3.69, 2.07, 1.38 times, all $P \leq 0.05$). Therefore, horses with severe gastric ulcers showed a more stressed pattern of behavior and HRV indicators, suggesting these may be reliable in determining severity of gastric ulcers in horses.

Introduction

Equine gastric ulcer syndrome (EGUS) has become increasingly prevalent with over 90% of racehorses in training and 36-53% of leisure horses affected (Hepburn, 2011). Horses are prone to ulceration due to the constant secretion of gastric acid into the stomach with risk intensified by activity level, diet, use of non-steroidal anti-inflammatory drugs (NSAIDs), environment, and stress. Equine gastric ulcer syndrome is traditionally confirmed with gastroscopy; however, occurrence is also linked to several clinical signs including poor performance and haircoat as well as abdominal pain and behavioral changes (Hepburn, 2011). Vatistas et al. (1999) reported gastric ulceration in 82% of a group of horses in race training, in which 39% also displayed clinical signs consistent with gastric ulceration including decreased body condition score. Another study by Murray (1992) evaluated abdominal discomfort in 111 horses and determined gastric ulceration to be the primary cause of pain in 28% of the study's population.

In addition to gastroscopy, behavior and stress responses have also been investigated to assess pain associated with gastric ulceration. Such responses have resulted in elevated heart and respiratory rates, increased body temperature and blood pressure as well as facial expressions and several other behavioral signs (Rietmann et al., 2004). Malmkvist et al. (2012) measured fecal cortisol metabolites and behavior in response to a novel object test; resulting in horses with severe ulceration to have an elevated cortisol concentration, paw more frequently, and eat quicker compared to control horses. Behavioral indicators of stress and occurrence of stomach lesions have also been evaluated in other livestock. Dybkjaer et al. (1993) found an association between frequency of oral behaviors, such as manipulating the bellies, ears and tails of pen-mates, and risk of acute ulcers in swine. In addition, a relationship between abomasal damage and behavior was also prevalent in veal calves with the development of tongue-playing in their second ten weeks of life (Weipkema et al., 1988).

Although the correlation of behavior and stress response to gastric ulceration has been determined, little is known about the relationship between heart rate variability (HRV) and behavior as indicators of ulcer severity in horses. Heart rate variability may be a reliable indicator of EGUS as it is a tool used to evaluate the integrity of the autonomic nervous system, the interaction between physiologic state and autonomic control as well as the pathophysiology of diseases (Rietman et al., 2004). Heart rate variability describes the variation between consecutive heart beats via the time domain variables; standard deviation of beat-to-beat interval (SDRR) and root mean square of successive beat-to-beat intervals (RMSSD; Camm et al., 1996). Heart rate variability may also be assessed using power spectral analysis (PSA) in which high frequency (HF) reflects cardiac vagal innervation and low frequency (LF) represents the sympathetic activity, or stress response. In horses, HRV has shown a primarily vagal control over the equine heart yet may vary in parasympathetic activity encouraging HRV towards both measures of PSA (Kuwahara et al., 1996). As a known physiological stressor, HRV responses to the transport of horses resulted in an increase in SDRR and decrease of RMSSD, indicating a reduction in vagal tone (Schmidt et al., 2010). Rietmann et al. (2004) also observed a decrease in the HF component of laminitic horses, in which HF correlated strongly with pain behaviors, adrenaline and noradrenaline. Thus, both behavior and HRV may be used as non-invasive and clinically applicable tools to provide diagnostic information on pain levels.

The objective of this study was to examine the effect of severity of gastric ulceration on behavior and HRV indices associated with pain. It was hypothesized that horses with more severe ulcerative lesions may develop more stress-behavioral signs and show more sympathetic dominance over the autonomic nervous system. A better understanding of the effects of ulceration on behavioral and HRV indices is imperative to correctly assess pain levels, provide adequate analgesia and improve overall welfare to equids.

Materials and Methods

This research was approved by the Institutional Animal Care and Use Committee of Clemson University (IACUC Protocol #: 2020-013).

Horses

The horses are kept as part of the university teaching herd and were a part of a larger concurrent study investigating the influence of an oral polysaccharide supplementation in treating gastric ulceration (Svagerko et al., 2021). Ulceration was induced using a modified Murray method prior to the six-week trial in which ulcers remained throughout the current trial.

Six geldings and two mares (Quarter Horses and Thoroughbred; n=8) with a mean age of 15±4 years old were involved in this study. Horses were housed in 10’x12’ stalls for approximately 12h (0600 to 1830) and a mixed warm-season native grass pasture of sufficient yield for continuous grazing for the remaining time (1830 to 0600). While in stalls, horses were fed 0.5% BW long-stem hay in slow-feeder nets (©Orange Slow Feeder, WA, USA) twice daily at 0600 and 1200 with *ad libitum* access to water. In addition to forage, horses also received 0.25%BW in concentrate (Nutrena Triumph® Fiber-Plus, ©Cargill Inc., Holmesville, OH, USA) split into two feedings (0600 and 1800).

Gastric ulceration in horses was examined using a 3-meter-long flexible video endoscope (Custom 3m HD Gastroscope, Advanced Monitors). Horses were fasted for 16h, deprived of water for 5h, and sedated using either Xylazine, Dormosedan, Butorphanol, and/or Acepromazine (100 mg/ml – 0.5 ml/100 Kg BW) prior to the endoscopic examination (veterinarian protocol). Gastric ulceration was scored for lesions in the gastric nonglandular squamous mucosa and the glandular mucosa of the stomach by an experienced equine veterinarian (Table 1). Lesions were graded following the grading system for Equine Gastric Ulcer Syndrome (EGUS) recommended by the Equine Gastric Ulcer Council was used (Andrews et al., 1999), with EGUS score 0 representing a healthy intact epithelium without reddening or hyperkeratosis and score 4 for extensive and deep ulceration.

Table 4.1. Grading system for the squamous and glandular mucosa. Adapted from Andrews et al. (1999).

	Squamous mucosa	Glandular mucosa
Grade 0	The epithelium is intact and there is no appearance of hyperkeratosis	The epithelium is intact and there is no evidence of hyperemia
Grade 1	The mucosa is intact but there are areas of hyperkeratosis	The mucosa is intact but there are areas of hyperemia
Grade 2	Small, single, or multifocal (< 5) superficial lesions	Small, single, or multifocal (< 5) superficial lesions
Grade 3	Large, single deep or multiple (≥ 5) focal superficial lesions	Large, single deep or multiple (≥ 5) focal superficial lesions
Grade 4	Extensive lesions with areas of apparent deep ulceration	Extensive lesions with areas of apparent deep ulceration

Experimental design

Horses selected for this study were divided into two groups based on the severity of gastric ulceration: a severe ulcer group (S; n = 4), with at least one extensive lesion either in the glandular or non-glandular portion of the of gastric mucosa (scores 3-4), and a mild group (M; n = 4), with intact mucosa (scores 0-2). Score 2 and below represents an intact mucosa, but may have small lesions, and signs of reddening or hyperkeratosis (Nadeau and Andrews, 2009).

Horses were given a 24h period to rest after the third and final endoscopic examination of the previously mentioned oral polysaccharide supplement investigation. Behavior and HRV data were then collected within stalls across three consecutive days to account for day-to-day variability.

Behavior assessment and analysis

One video camera (Wyze Cam, ©Wyze Labs, Kirkland, WA) with a field of view of ~90%, was mounted to each 10’x12’stall to observe individual horse behavior. Behavior was recorded at three 2h time points per day: morning (MOR), afternoon (NOON), and evening (EVE). Recordings were later analyzed to score behavior using an ethogram of 21 behaviors (Table 2). Behaviors were analyzed from the videos in two 15min increments an hour, totaling nine hours of data per horse across the three days of data collection. All behaviors present during the 15min observation window were recorded as frequency of occurrence. All observations were conducted by a single observer.

HRV assessment and analysis

Heart rate (HR) of each horse was recorded for two hours daily (MOR, NOON, and EVE) across the three consecutive days. A HR monitor (Polar Electro OY, Kemple, Finland) was used for recording HR and HRV. The device consisted of an electrode belt with built-in transmitter and a wristwatch receiver. One electrode was located at the left shoulder, whereas the other one was located at the left thorax just behind the elbow joint. Warm water and SAFE-LUBE (Nasco, Fort Atkinson), a non-spermicidal multipurpose lubricant, were used to augment the contact between electrode and skin. Moreover, an accommodation period of approximately 30 seconds was allowed for each horse after fitting the electrodes to let the HR settle within a normal range. The polar device records the changes in electrical potential to detect average, minimum, and maximum HRs in addition to the beat-to-beat (RR) intervals.

Table 4.2. Ethogram used to score horse behavior.

Behavior	Definition	Reference
Standing	Standing relaxed	Malmkvist et al. (2012); Torcivia and McDonnell (2021)
Eat	Chewing food; head may be lifted from food for short periods while chewing continues	Bulmer et al. (2015)
Drink	Mouth in contact with water dispenser; Swallowing water	Malmkvist et al. (2012); Young et al. (2012); Winskell et al. (1996)
Head nodding	Rapid nodding head up and down in small movements	Ellis et al. (2014); Reid et al. (2017)
Head shaking	Rapid shaking head from side to side	Ellis et al. (2014); Reid et al. (2017)
Jaw movements	Conservative or atypical jaw movements (not foraging, chewing, or yawning)	Torcivia and McDonnell (2021); Reid et al. (2017)
Tongue activity	Extraneous movement of the tongue in and out of the mouth	Malmkvist et al. (2012)
Locomotion	Intentional movement of more than 2-3 steps	Martenson et al. (2009)
Restlessness and agitation	Changing major activity (foraging, standing rest, standing alert) more frequently than expected	Torcivia and McDonnell (2021); Reid et al. (2017)
Tail swishing	Moving tail suddenly from side to side	Torcivia and McDonnell (2021); Reid et al. (2017)
Kick abdomen	Flexing a hindlimb, directing the hoof or stifle toward the abdomen	Torcivia and McDonnell (2021)
Looking back at chest or abdomen	Glancing or gazing at a particular area of the body	Torcivia and McDonnell (2021)
Vocalization	Audible vocalization, call	Ellis et al. (2014); Reid et al. (2017)
Pawing	Continuous kicking/scraping with front leg	Ellis et al. (2014); Reid et al. (2017)
Urination or defecation	Passing urine or feces	Gleerup and Lindegaard (2016); Reid et al. (2017)
Scratching/Autogrooming	Nibbling, nuzzling, and/or biting at an area of the body, or rubbing one part of the body to another or against an object	Torcivia and McDonnell (2021)
Biting at side/Swatting	Swinging the head and neck, batting at a particular area of the body	Torcivia and McDonnell (2021)

Stretching	Raising and pulling head caudally, with the back curved ventrally; Extending forelimbs cranially, shifting weight onto hindlimbs with shoulders lowered towards ground; Extending hindlimb caudally	Torcivia and McDonnell (2021)
Kicking	Lifting and extending one or both hindlimbs caudally	Torcivia and McDonnell (2021); Reid et al. (2017)
Stomping	Suddenly flexing and then extending a limb, sharply striking the hoof against ground	Torcivia and McDonnell (2021)

It stores them into a digital form, as the wristwatch receives and sorts the data from the transmitter. The data were downloaded from the receiver via a polar interface (Polar ProTrainer Equine edition) to the personal computer. HRV was then analyzed with Kubios HRV software, version 2.2 (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland, 2014).

Data were detrended to remove trend components, and artifact correction was made (Schmidt et al., 2010) following established procedures (Tarvainen et al., 2002). The mean HR was measured in beats per minutes. For the analysis of HRV, the most informative time and frequency domain measures were obtained: the SDRR (milliseconds; Manteca and Deag, 1993) and RMSSD (milliseconds) were calculated. The RMSSD is determined by calculating the difference between consecutive RR intervals before squaring and summing them; the values are then averaged, and the square root is obtained (Stein et al., 1999). The RMSSD and SDRR are used to estimate HF beat-to-beat variations that represent mainly vagal regulatory activity (Stein and Kleiger, 1999, Von Borell et al., 2007). The RR interval data were subjected to PSA using the autoregressive model (Bernasconi et al., 1998). This method was chosen instead of fast

Fourier transform because it allows the comparison of short-time RR intervals (Kuwahara et al., 1996), which is advantageous for our study because of short-time RR recording. The LF and HF were calculated in normalized units (nu). LF nu and HF nu represent the relative value of each power component in proportion to the total power of the spectrum (Pagani et al., 1986, Camm et al., 1996), which allows the comparison of different measurements and subjects (Bernasconi et al., 1998). The LF/HF ratio represents the SVB. Frequency component thresholds were set at 0.01-0.15 Hz for LF and 0.15-0.5 Hz for HF (Rietmann et al., 2004).

Statistical analysis

The assessor was trained by observing, describing, and distinguishing different behavioral responses that were videotaped during different procedures of the study. Twenty-five percent of video clips were assessed twice by the same observer; once intra-observer reliability reached >90%, assessor scored the remaining clips. HR, HRV parameters, and frequency of occurrence of observed behaviors for each horse during each time point through the 3 days of observation were obtained. Data were normally distributed as tested by the Shapiro-Wilk's test ($P > 0.05$) and a visual inspection of their histogram, normal Q-Q plots, and box plots showed that the data were approximately normally distributed for all the treatments. Descriptive statistics were used for the analysis of the data as follows: means and standard errors of the means and analysis of variance with treatment as the main effect were used to compare among different treatment groups. Statistically significant effects were further analyzed, and means were compared using Tukey's honestly significant difference multiple comparison procedure.

The statistical significance level was set at P ≤ 0.05. Statistical analyses were performed using R software (version 3.3.1), package "stats" (R Core Team, 2013).

Results

Behavior

The most frequently required behaviors observed from the ethogram were eating, urination and defecation, abdominal kicking, tail swishing, pawing, head shaking, looking back at chest or abdomen, restlessness and locomotion (Table 3). Severity of ulceration was associated with behavior in which the S group displayed a significant increase (P<0.05) in occurrence overall compared to the M group. Significant increases in behavior occurrence were also demonstrated within each of the observation times (MOR, NOON and EVE), with a trend for locomotion during the NOON observation (P=0.06). No differences in day-to-day behavior were detected.

Table 3. Frequency of behavior occurrence within the M and S ulcer groups overall and during the MOR, NOON, and EVE observation timepoints.

	Eat	Uri/Def	Abd kick	Tail swish	Paw	Head shake	Look back	Tongue act	Restless	Locomotion
					Overall					
Mild	3.84	0.48	1.32	3.69	1.38	0.83	1.27	2.07	4.67	5.39
Severe	12.06*	1.74*	5.26*	10.00*	6.87*	5.66*	4.75*	10.35*	16.59*	14.10*
					MOR					
Mild	4.66	0.66	1.23	3.66	1.03	0.69	1.03	1.41	4.36	3.99
Severe	13.63*	2.03*	5.33*	8.66*	7.36*	4.63*	3.69*	9.66*	16.36*	14.36*
					NOON					
Mild	2.88	0.36	1.03	2.84	1.12	1.03	1.36	1.96	5.63	5.23
Severe	9.66*	1.30*	4.23*	7.66*	5.22*	5.68*	4.56*	10.36*	15.45*	7.58
					EVE					
Mild	3.98	0.42	1.69	4.58	1.98	0.78	1.42	2.85	4.03	6.96
Severe	12.88*	1.88*	6.23*	13.69*	8.03*	6.66*	5.99*	11.02*	17.96*	20.36*

*Values differ within a column and timepoint ($P \leq 0.05$).

Heart rate variability

All overall HRV indices significantly differed between the two ulcer severity groups (Table 4). Mean overall HR was observed to be significantly higher in the S group than the M group (63.32 and 51.08, respectively; P<0.05). In addition, the overall LF/HF ratios within the S group were significantly greater than the M group (5.00 and 3.25, respectively; P<0.05). The S group also displayed significantly reduced SDRR and RMSSD overall values, compared to the M group (155.6 and 199.0; 39.73 and 52.57, respectively; P<0.05). Similar to the overall results, identical significances were seen within each observation time (MOR, NOON, and EVE). No differences in day-to-day HRV indices were observed.

Table 4. HRV parameters within the M and S ulcer groups overall and during the MOR, NOON, and EVE measurement timepoints.

	Mean HR	SDRR	RMSSD	LF/HF (%)
Overall				
Mild	51.08	199.0	52.57	3.25
Severe	63.32*	155.6*	39.73*	5.00*
MOR				
Mild	47.71	221.2	61.73	2.35
Severe	66.09*	143.0*	37.67*	5.48*
NOON				
Mild	45.10	214.9	57.66	2.22
Severe	51.44*	184.0*	42.70*	3.79*
EVE				
Mild	45.06	232.1	63.09	2.44
Severe	62.22*	139.9*	38.81*	5.74*

*Values differ within a column and timepoint ($P \leq 0.05$).

Discussion

Even though gastroscopy is the reliable mechanism for diagnosing EGUS, behavior and HRV indices have been considered as potential alternatives in assessing pain, and thus ulcer severity. In this trial, behavior and HRV indices were analyzed in horses with varying gastric ulcer scores. It was the aim of the study to investigate these parameters in their association with pain in response to severity of gastric ulceration.

Although no true evidence was presented in that behaviors are a result of pain induced by ulceration, an association of behavior frequency and ulcer score was observed. Horses with severe ulcer scores (3-4) demonstrated an increase in eating bouts as well as displayed a greater frequency of behaviors such as abdominal kicks, tail swishing, tongue activity, restlessness and pawing than mildly ulcerated (scores 0-2) horses. It was expected for horses with severe lesions to develop more stress-behavioral signs. Previous studies have shown an increase in similar behaviors among several animal species at risk of gastric ulceration (Malmkvist et al. 2012; Dybkjaer et al., 1993; Weipkema et al., 1988). In a preceding equine study, an association was described between more frequent and shorter eating bouts and increased pawing with the presence of severe glandular ulcers (Malmkvist et al. 2012). Likewise, Nicol et al. (2002) found similar results with a higher incidence of squamous gastric ulcers in crib-biting foals. In the current study, despite the increase in behaviors of the severe ulcer group, horses did not exhibit any stereotypic behaviors potentially due to the consistent forage access (Houpt, 1981). Therefore, constant access to forage in a stalled environment may inhibit the risk of stereotypical behavior development in horses with EGUS. The camera position and field view of 90%, however, served as a leading to possible missed behaviors in the remaining 10%.

Comparable to behavior, an elevated heart rate was observed with increasing ulcer severity. The increase in heart rate also corresponded with a significantly reduced SDRR and RMSSD and increased LF/HF ratio, previously reported as indicators of anxiety and stress (Reid et al., 2017; von Borell et al., 2007). In stressful situations, the parasympathetic nervous activity decreases to accommodate a higher sympathetic activity, allowing animals to react accordingly in a biological manner (Bachmann et al., 2003). The difference in HRV values between the two ulcer groups suggests there may be a shift toward sympathetic dominance and potentially a physiological response to pain associated with EGUS. Reid et al. (2017) observed similar results in horses with induced anxiety, determining a relationship between an increase in heart rate and decreased SDRR. The previous study also investigated induced pain and observed an increase in SDRR, potentially differing from the current study as pain was induced by a neck pinch for only five minutes as opposed to constant presence of gastric ulcers. Additionally, in a pain assessment of horses suffering from laminitis, Reitmann et al. (2004) determined an increased LF was associated with higher levels of adrenalin. Significant shifts in HRV have also been demonstrated in horses with grass sickness and atrial fibrillation (Perkins et al., 2000; Kuwahara et al., 1998).

Conclusion

This study presented a strong association in behavior and HRV indices to gastric ulcer severity in horses. Horses with an ulcer score of 3 or 4 showed a more stressed pattern of behavior and HRV indices thus providing a potential method in determining severity of lesions without invasive procedures such as gastroscopy. In addition, management techniques may be altered to appropriately diminish the risk of EGUS and physiological consequences. A thorough understanding of the effects of EGUS on behavioral responses and HRV parameters is important to correctly assess pain levels, provide adequate analgesia, and improve overall welfare.

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CONCLUSION

The scope of this research has provided valuable insight for the equine community in efforts to improve pasture management techniques for horses and gastric ulceration detection. Maneuvering required maintenance elements was deemed influential on distributing horse location and behavior in a pasture environment. Horses exhibited the tendency to stay near the feeding element more often compared to shelter or water as it was moved around a pasture. The follow-up investigation on feeding frequency and mechanism also shed light on horse activities where traditional twice daily feeding by a human showed greater horse presence around a feeding location than shelter or water. The drive that horses exhibit to consume a concentrate feed was evident, as the feeding element was most influential on horse location and behavior in both trials. The investigation on the association of behavior and HRV indices to gastric ulcer severity in horses may have also provided a potential method in determining severity of lesions without invasive procedures such as gastroscopy.

While further research is necessary to validate the proposed grazing techniques, this author can encourage producers to consider providing supplemental feed twice daily in frequently moved locations within a pasture setting. This technique may encourage horses to centralize their activities around the feeding location, allowing other areas of the respective pasture time to rest and replenish. The ease of simply moving feeding locations will hopefully alleviate the management of horses at pasture as a minimal amount of extra time to move feed pans or buckets and no added costs of fencing or other materials are associated with the technique.

Future research expanding the use of pasture management techniques specifically for horses is warranted. Such research may include the effects that maneuvering required maintenance elements have on equine grazing behavior and location in varying seasons. Available plant species within different growing seasons may impact horse foraging preferences, potentially affecting location within a pasture and behavior. Another avenue of future research could evaluate the effects that these proposed grazing management techniques have on forage and soil health. While visual assessment can be helpful, it would be critical to determine the direct environmental effects associated with moving feed, shelter, and/or water before recommending this management technique to producers. Field studies followed up with surveys completed by producers may also be enlightening in the validation of required maintenance elements as pasture maintenance tools. Trials by producers may determine the practicality of application on a daily basis on a variety of facility sizes. In addition, since supplemental feed was the most influential element in both trials, evaluating alternative supplemental feed sources may allow for the technique to be more applicable for producers nationally and internationally.

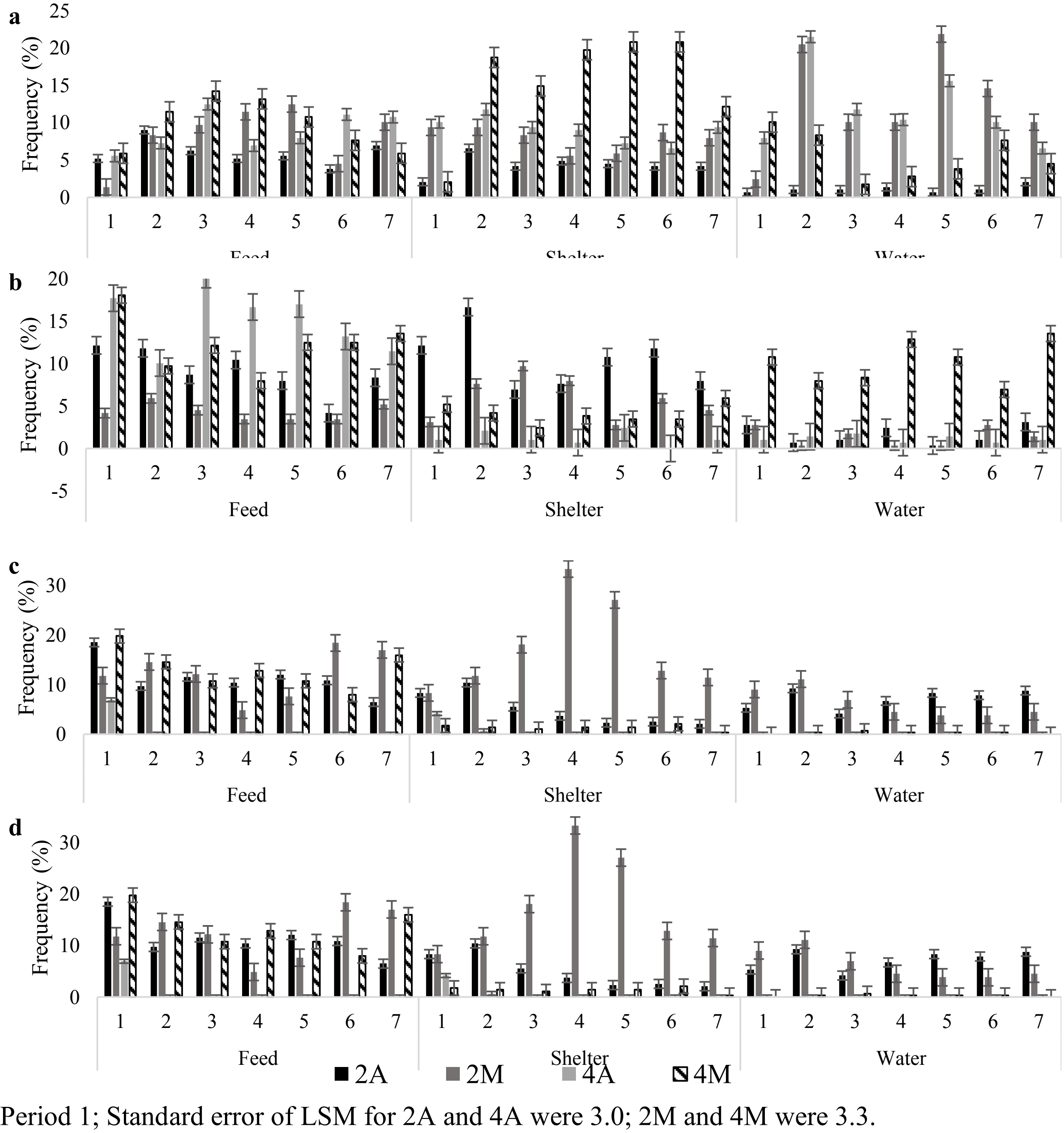
Overall, there is a large opportunity for further research and development of maneuvering required maintenance elements, with emphasis on feed, within an equine pasture environment as a management method. There is evidence this technique may benefit equine producers. In addition, the association between behavior and HRV indices may be useful to producers on management of EGUS in horses.

APPENDICES

Appendix A and B include further results detected in Chapter III. Appendix C does not align with the direct focus of the dissertation; however, it is in preparation for submission to Clemson University Land Grant Press.

Appendix A

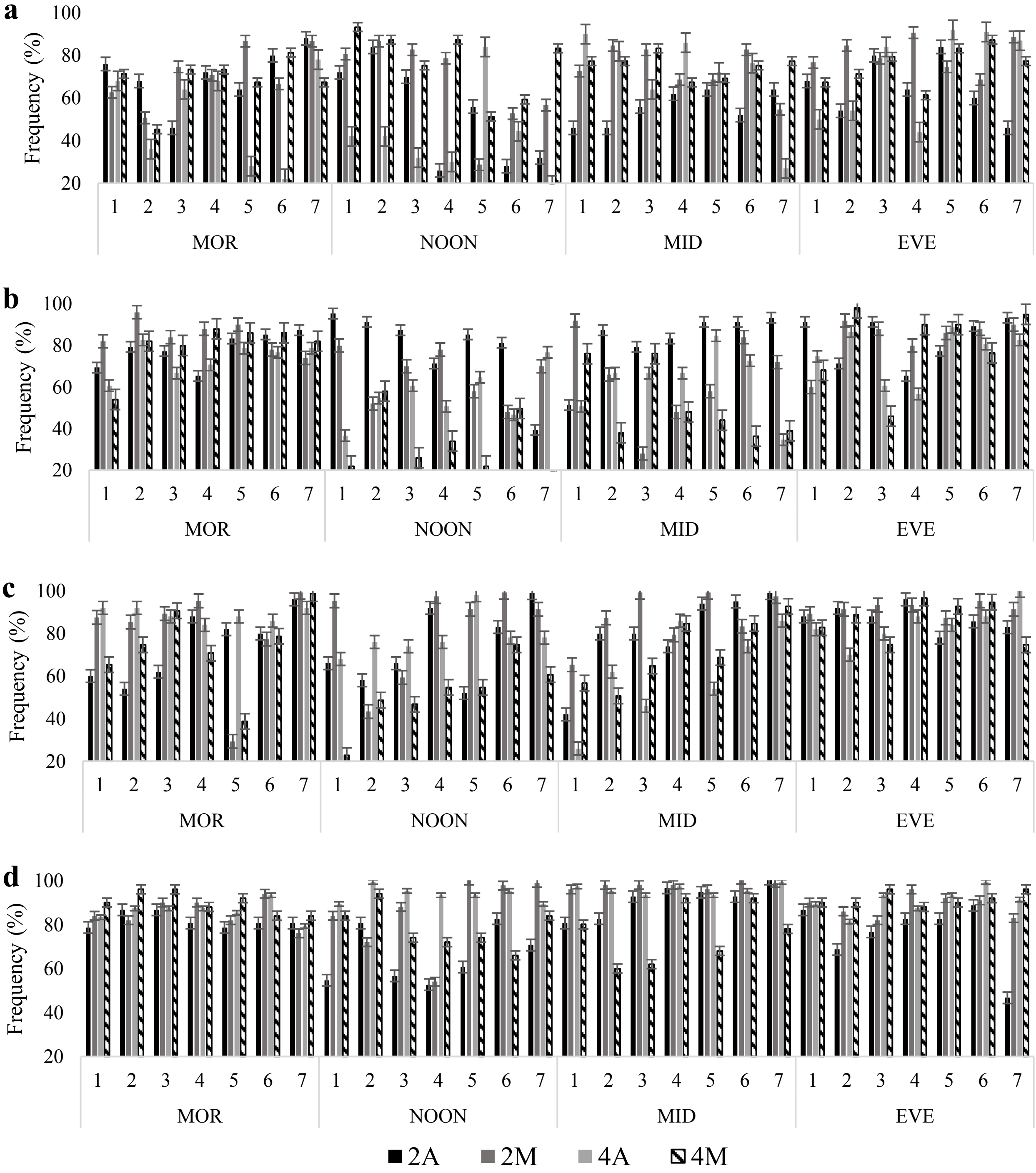
Main effect interaction of day (1-7), period and treatment with location of horse in pasture within 23 m of feed, shelter or water. Data are presented as percent LSM with SEM bars.



[a] Period 1; Standard error of LSM for 2A and 4A were 3.0; 2M and 4M were 3.3.
[b] Period 2; Standard error of LSM for 2A and 4A were 3.3; 2M and 4M were 3.0.
[c] Period 3; Standard error of LSM for 2A were 2.6; 2M ad 4M were 3.3; 4A were 3.7.
[d] Period 4; Standard error of LSM for 2A and 4A were 3.0; 2M and 4M were 3.3.

Appendix B

Main effect interaction of period, day, time and treatment on Grazing behavior across 4, 7-day periods and behavior observation of MOR, NOON, MID and EVE. Data are presented as percent LSM with SEM bars.



[a] Period 1; Standard error of LSM for 2A and 4A d 1-7 were 6.9; 2M and 4M d 1-4 were 6.0; 4M d 5-7 were 5.5.

[b] Period 2; Standard error of LSM were 8.5 except for 2A d 6 and 7, 4A d 1 and 4M d 7 were 11.9 and 4M d 6 were 7.1.

[c] Period 3; Standard error of LSM for 2A d 1-5 were 10.1; 2A d 6 were 8.5; 2A d 7 were 14.1; 2M and 4M d 1-7 were 10.2; 4A d 1-7 were 10.1.

[d] Period 4; Standard error of LSM for all LSM were 5.6 except for 2M d 6 and d 7 were 4.7 and 7.8, respectively.

Appendix C

PRODUCING, PACKAGING, AND MARKETING SUSTAINABLE MANURE-BASED COMPOST ON A UNIVERSITY EQUINE CENTER

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Abstract

Animal waste has been converted to compost for several decades as a method of disposal and environmental resource. The goals of this project were to: 1) convert horse waste to compost, 2) sustainably package the end-product and 3) practice compost marketing techniques. Equine waste was collected and stored in four wooden-pallet bays from individually stalled mature horses at the Clemson University (CU) Equine Center in Pendleton, SC. Waste consisted of a manure-based mixture (MBM): feces, urine, long-stem forage and wood-shavings. Two treatment bays (TRT) were covered with corrugated sheet metal and three one-inch PVC pipes placed at the base of the MBM with 0.32 cm holes for aeration (Fig. B.1). Two control bays (CTRL) were left uncovered with no aeration. Temperature of all piles was taken biweekly; post-recording, TRT piles were manually turned. Two composite samples were taken from TRT and CTRL piles at three and six weeks and submitted to CU Regulatory Service Lab (RSL) for standard analysis including: percent moisture, total nitrogen (N), total carbon (C), C:N, pH, organic matter (OM) and soluble salts (EC) and shown in Tables B.1 and B.2. Analyses revealed CTRL and TRT piles only reached recommended nutrient levels such as N, EC and pH, as defined by the CURSL. Thus, more than six weeks was required for compost to reach an

adequate soil amendment quality. A linear model was developed that related nutrient levels of compost to the fixed effect of treatment. An ANOVA was used to determine if the fixed effects were significant, followed by Fisher's Protected LSD to compare means. Results determined differences ($P < 0.05$) in a variety of nutrients between control and treatment piles across Piles 1 and 2 over the three- and six-week trial periods further seen in Table B.3. Once compost reached a consistent 55ºC, it was packaged into repurposed feed bags and sealed using an industrial sewer. The poly-weave bags maintained compost moisture content and simplified product distribution. The finished product was then advertised on the CU Equine Center Facebook page and CU Marketplace where orders were received and fulfilled. This marketing technique was deemed successful as all available compost was sold upon one month of initial advertisement. Thus, equine manure from a university facility can be converted to compost, packaged sustainably and used as a quality soil amendment.

Figure C.1 Image of control and treatment (covered and three, one-inch pipes with 0.32 cm holes for aeration placed under MBM) wooden-pallet bays, respectively.

Table C.1 Laboratory analysis for both control and treatment Pile 1 at three and six weeks. Data are presented as raw values.

Nutrients	Control		Treatment		Recommendation*
	3wk	6wk	3wk	6wk	
N (dry basis%)	1.8	1.6	1.3	1.8	0.5-2.5
C (dry basis%)	49.4	48.8	43.2	46.7	54.0
C:N	22.7	32.2	32.9	27.2	20.0 - 30.0
OM (dry basis%)	90.0	89.7	83.6	82.0	50.0 - 60.0
EC (mmhos/cm)	1.5	1.0	3.2	3.2	1.0 - 10.0
pH	7.8	7.7	8.0	8.3	5.0 - 8.5
Moisture (%)	76.4	76.7	64.0	65.1	40.0 - 50.0

*Recommendations from Clemson University Regulatory Services lab were utilized as a guideline to determine when compost was deemed appropriate to use as soil amendment.

Table C.2 Laboratory analysis for both control and treatment Pile 2 at three and six weeks. Data are presented as raw values.

Nutrients	Control		Treatment		Recommendation*
	3wk	6wk	3wk	6wk	
N (dry basis%)	1.9	1.7	1.0	1.4	0.5-2.5
C (dry basis%)	47.2	46.1	46.8	44.0	54.0
C:N	25.5	28.5	48.8	34.7	20.0 - 30.0
OM (dry basis%)	85.8	84.5	81.4	78.5	50.0 - 60.0
EC (mmhos/cm)	3.5	3.2	4.0	4.2	1.0 - 10.0
pH	8.3	7.9	8.3	7.9	5.0 - 8.5
Moisture (%)	55.7	69.9	55.4	57.2	40.0 - 50.0

*Recommendations from Clemson University Regulatory Services lab were utilized as a guideline to determine when compost was deemed appropriate to use as soil amendment.

Table C.3 Nutrient analysis across control and treatment Piles 1 and 2, and the three- and six-week timepoints within the trial. Data are presented as LSM ± SEM.

Nutrient	Control	Treatment	SEM	p-Value
N (dry basis%)	1.7^{a}	1.4^{b}	0.07	0.003
C (dry basis%)	47.9^{a}	45.1^{b}	0.9	0.05
C:N	27.2^{a}	35.9^{b}	2.4	0.03
OM (dry basis%)	87.5	81.4	1.0	0.001
EC (mmhos/cm)	2.3^{a}	3.7^{b}	0.4	0.04
pH	7.9	8.1	0.1	0.3
Moisture (%)	69.7	60.4	3.0	0.06

^{ab}LSM within not connected by the same letter are significantly different ($P < 0.05$).