Methods of Restricting Forage Intake in Horses

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Abstract

Horses have evolved to be hindgut fermenters, requiring small amounts of forage to be consumed throughout the day [1]. However, due to the recent increase in equine obesity [2–4], it has become necessary to restrict the amount of feedstuffs a horse consumes, often resulting in a restriction of forage intakes. In order to maintain a healthy gastrointestinal system, management strategies should attempt to replicate a horse's natural foraging habits. The objectives of the following studies were: 1) to investigate the effectiveness of decreasing pasture forage intakes via use of a grazing muzzle, and whether the effectiveness could be altered by grass morphology and palatability, 2) to investigate the effectiveness of "slow-feed hay nets" at increasing time to consumption of a preserved forage meal in stalled horses and 3) to observe the effects of increased time to consumption of daily rations on the post-prandial metabolic response. To determine objective 1, a two-year study was designed where four horses were used in a Latin square design in Year 1, while 3 horses were used in a completely randomized design in Year 2. Horses were grazed for 4 hours on monoculture plots four days per month for four months. Initial herbage mass and residual herbage mass measurements were taken to determine forage intakes. For objective 2, 8 horses were used in a replicated Latin square design, with 2 horses assigned to a treatment at a time. There was a control (C) of feeding hay on the ground, as well as three treatments: small-opening net (SN), medium-opening net (MN) and large-opening net (LN). Horses were allowed 4 h to consume their hay meal. Time to consumption and dry matter intake rate were measured using a stopwatch and any orts remaining after the 4 h were collected and weighed. To estimate objective 3,

8 overweight horses were enrolled in a randomized complete block design. Horses were blocked by bodyweight, BCS, and gender. Horses were fed a control diet of hay at 2% BW for a period of 10 days, and were then switched to a restricted diet of hay fed at 1.08% and ration balancer fed once daily at a rate of 0.001% BW. Horses were assigned to one of two treatments: hay fed off the floor (FLOOR) and hay fed in a small-opening hay net (HN). Serial 24 h blood samples were taken on day 0, when horses were still on baseline diet, as well as days 14 and 28. Plasma glucose, insulin, cortisol, and leptin values were estimated.

Results of objective 1 found that grazing muzzles were effective at decreasing pasture intakes by 30% ($P < 0.0001$). Species had no effect on intakes in Year 1 ($P = 0.27$), but did impact intakes in Year 2 ($P = 0.042$). Results of objective 2 found that SN and MN were effective at increasing total time to consumption ($P < 0.0001$) compared to horses on the control and LN, more closely mimicking a horses' natural foraging behavior. Results of objective 3 found that hay nets decreased overall stress of horses on a restricted diet ($P < 0.05$), however length of sampling and weight loss had a larger impact on post-prandial metabolite. Horses on day 28 of the trial had higher average glucose, insulin and cortisol values, as well as lower AUC cortisol. Increasing time to consumption of forages is a healthy method of decreasing body weight while maintaining healthy post-prandial metabolite values.

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CHAPTER I: LITERATURE REVIEW

Equine Nutrition Overview

Horses have evolved as hindgut fermenters, subsisting on large amounts of low to mid-quality forage consumed throughout the day [1]. Equines have developed an enlarged cecum, also referred to as the hindgut, which is necessary for fermentation of forage structural carbohydrates by the resident microbial colonies. While the horse itself does not produce the enzymes necessary for digestion of some of the plant components it consumes, including cellulose and hemicellulose, the hindgut microbes possess the enzymes necessary to digest the plant structural carbohydrates, creating end products useful to the horse. Cellulose, and the digestible cell wall components bound by cellulose, are an important source of fiber to the horse, making cellulolytic microbial fermentation, an important process in nutrient acquisition. The microbiota that reside in the hindgut are able to produce nutrients, such as volatile fatty acids (VFA's), that are nutritionally useful to the host horse. VFA's can provide up to 80% of a horse's daily energy requirements, creating an important symbiotic relationship between the horse and microbes [1],[5].

The importance of forage in equine nutrition is often underestimated by many horse owners and managers; however, many researchers agree forage is the most important feedstuff in a horse's ration. Nutritionists recommend that forages comprise at least 60% of the horse's daily ration, allowing for proper fiber intakes and gut health [6]. The ingestion of fibrous feedstuffs increases the amount of chewing performed by the horse, resulting in production of salivary juices which can act as buffers in the equine stomach. This is important especially in horses fed concentrates, as it can help neutralize the pH of the stomach, decreasing the risk of development of ulcers [4–6].

When horses are fed large amounts of concentrates in a single meal, it is more likely that a large amount of undigested starch will reach the hindgut, leading to alterations in microbial fermentation [4,7,8]. This can in turn have negative effects on fecal pH and lead to other deleterious effects such as colic and laminitis [4,7,9]. Both colic and laminitis are severe and sometimes fatal diseases that occur in the equine population, with colic representing approximately 50% of equine fatalities [13]. Additionally, increased occurrence of meal feeding has been linked with the increased occurrence of stomach ulcers, which are more pronounced in performance horse populations. It is estimated that approximately 81-93% of racing Thoroughbred’s either currently or have had stomach ulcers, and approximately 58-66% of hunter, jumper and dressage horses are also afflicted [10,11].

It is thought that the high occurrence of ulcers in these horses can be attributed to the large amount of concentrates fed and relatively low amount of forages fed. With performance horses, hay is usually only fed in small quantities, in order to prevent the formation of hay belly, and to decrease the amount of water weight that has to be carried, which can negatively affect athletic performance [16]. Often, it is more feasible to meet energy requirements of high-performing horses with moderate to large amounts of concentrates, compared to the large amounts of forage it would take to meet those same requirements. Additionally, it has been found that increased amounts of forage led to increased water intake [32,33] along with increased amounts of water present in the gastrointestinal tract [19]. Increased amounts of water are undesirable for performance horses as it leads to increased weight which may decrease performance. In a study investigating the effects of forage intake on bodyweight and performance, it was found that an all-hay diet led to increased bodyweight, maximum heart rate, and mean recovery heart rate when compared to diets that had a lower percentage of hay [19]. However, all horses were meal-fed, and presumably consumed each meal very quickly. It has not been investigated if prolonged hay consumption would lead to the same increases in bodyweight and heart rates, or if the effects of forage would be mitigated by decreased rates of passage through the digestive tract.

Increasing the amount of forage in the ration of performance horses would likely help decrease the occurrence of ulcers, however it must be achieved without a negative effect on their performance. A causative factor of ulcers that is coupled with low forage intake is the fact that horses do not have a constant flow of salivary buffers into the stomach to help neutralize the effects of chemical digestion. Unlike humans and many other animal species, saliva production can only be stimulated by the mechanical action of chewing [20].

There are also some instances where higher hay intakes and gastrointestinal water availabilities would be beneficial to performance horses. With endurance horses, where the horses are expected to perform for extended periods of time without any feed or water, greater amounts of water in the gastrointestinal tract would be beneficial [21]. A meal high in forage prior to performance should give a constant supply of nutrients and water. A lack of water, or dehydration, can result in high core temperatures and decreased cardiac output [22], leading to lower performance. Significant amounts of electrolytes can also be lost via sweat during a workout or performance. Warren (1999) found that an increase in total and soluble fiber intake led to decreased chances of dehydration during an endurance event [21]. Danielsen et al. (1995) found that an increased hay diet led to increased total feed and water consumption when compared to a limited roughage diet [18], leading the researcher to conclude that a high hay diet would be beneficial for horses that were involved in any type of endurance exercise.

The ingested forage or concentrate moves from the stomach to the small intestine for further digestion and absorption in a fairly short period of time, approximately 2-6 h [23]. Nutrients are digested and absorbed during their transit through the small intestine, with the majority of fiber and forage contents being chemically untouched until they reach the large intestine. In the cecum, the remaining digesta, mostly fibrous feedstuffs, are then fermented by the microbial colonies present. Unabsorbed material spends the greatest proportion of time in the large intestine, approximately 75-85% of total transit time [14,15]. In order to maximize digestibility of any feed, the retention time in the digestive tract must also be maximized [26]. This in turn increases microbial activity along with increased absorption of water from the gastrointestinal tract.

While concentrates are usually more digestible than forages, the rate of passage of forage is typically quicker than that of concentrates, due to increased bulk of the forage ingested in a short period of time. Stevens and Hume [27] found that diets of long-stemmed hay increased the rate of passage through the hindgut, most notably the particulate digesta passage rate, when compared to pelleted diets that were much smaller in size. However, the smaller size of the pelleted meal led to decreased salivary buffer production, and when fed in combination with longer-stemmed hay, the increased passage rate led to an increase in the amount of undigested starch that reached the hindgut. Undigested starch that reaches the hindgut is a causative factor in the pathology of both colic and laminitis, as the undigested starch leads to disruptions in the microbial ecology of the hindgut [27].

Several other studies have supported the theory of increased particle size decreasing passage rate, or mean retention time (MRT) through the gut. Shortest MRT of fiber has been associated with shorter fiber lengths when comparing silage chopped at different lengths [28]. Fiber chopped at 6.8 cm decreased MRT when compared to fiber chopped at 29.5 cm [18, 19]. In a study comparing transit times of grass hay and alfalfa, it was found that there was no difference in transit time, but increasing the proportion of alfalfa in the ration led to increased passage rates [23]. The authors attributed this effect mostly to the differences in fiber lengths of the forages, with alfalfa having shorter fiber length compared to grass hay. Another study investigating the effect of ryegrass/ timothy mix hay cut at 18 cm and 5.3 cm on digestibility found that fiber length did not impact MRT or digestibility [23]. However, fiber length is not to be confused with particle size. As increased particle size significantly increases MRT, opposite of the findings of Morrow et al. (1999) and Moore-Colyer at al. (1992).

Another possible factor affecting passage rate and MRT is the water-holding capacity of the plant. Fibrous feedstuffs, such as hay and fresh forages, have been found to increase the horse's water holding capacity in the gastrointestinal tract [21].

Researchers have cited this as being due to the gastrointestinal tract acting as a fluid and electrolyte reservoir [23, 24], as well as maximizing the water intake of horses [24, 25]. Grass hay has increased fiber content compared to alfalfa hay, giving it the ability to absorb more water. Cuddeford et al. (1992) estimated that grass hay contained approximately 260 g/kg of hemicellulose, a type of fibrous carbohydrate that has been correlated with increased water intake, while alfalfa had only 59 g/ kg [32].

While there are many variables that are important in determining rate of passage and MRT of feedstuffs through the gastrointestinal tract, the most important variable is likely the hay to concentrate ratio of the diet. Increases in the hay: concentrate ratio led to decreased MRT and increased passage rates, leading to decreased digestibility [33]. This was due to larger feed volumes coupled with higher water intake, increased saliva production, and increased alimentary secretions [28–30]. However, a balance must be met between amount of forage in the diet and digestibility of diet since large amounts of forage are required for optimal hindgut performance. It is recommended that to avoid gastric disturbances, limiting concentrates to less than 2.7 kg (in this case oats were used, considered as one of the safer concentrates to feed for horses) [37], and avoiding rations with more than 2-3 g/kg BW of starch will provide adequate amounts of energy, while avoiding starch overload [38].

As mentioned before, for most performance horses, it is not feasible to subsist solely on forage to meet their energy requirements. These horses will generally be fed concentrates in order to increase their daily energy intake, enabling them to perform more intensive exercises at higher levels while maintaining body weight and condition.

Kronfeld (1996) found that when fed hay and oats (50:50 mixture), both energy intake and digestibility were increased when compared to a diet of hay only (100:0) [39]. It was previously recommended (NRC, 1989) that concentrates should form 70% of the equine diet for growth and 65% of the diet for intense work; however, the dangers of increased concentrate feeding were not well understood [40]. Recently, the recommendation has been revised, and the percentage of concentrate reduced to be less than 40% of the total daily ration. Hudson (2001) recommended daily intake of oats to be fed at levels less than 2.7 kg/ d, as horses fed over this level were at an increased risk of colic [37]. Reeves (1996) also found that an increased intake of concentrates containing whole grain corn enhanced the risk of colic by 3.4% per ingested kilogram [41].

Several studies have tried to isolate the exact cause of colic, as it is extremely detrimental to the horse population. Researchers have found that there is no single cause of colic, but rather a multifactorial source of predisposing factors. It is widely agreed upon that increasing the portion of roughage (i.e. forage) in the diet has a positive impact and decreases the risk of a horse having a colic episode [10, 31, 36–38]. In a study by Hudson et al. (2001), it was found that stalled horses, horses with decreased pasture access, and horses with increased concentrates intakes all had elevated risk of colic when compared to horses that had ad libitum access to pasture, or pasture was their sole food source [37]. Tinker et al (1997) reported that if horses had access to multiple pastures, they were at a decreased risk of colic compared to horses with no pasture, and that feeding large amounts of concentrate greatly increased the risk of colic [25]. Cohen et al. (1999) also found that pastured horses had a decreased risk of colic, but horses fed poorer quality hay or experienced any abrupt change in type of hay had an increased risk of colic [44].

Additionally, other factors such as feeding hay off of the ground [45], feeding a diet with an imbalance of roughage to concentrate [26], and feeding horses from round bales [37] all increased a horse's risk of colic. Because of this, it is important to determine a horse's nutritional needs, and what feeding regimen best fits those needs, while acknowledging the importance of including forage as a large part of a horse's daily ration.

Equine foraging behavior

Horses are selective grazers, preferring certain types of forages and differentiating between different parts of a plant [1]. In a study looking at the differences in grazing behavior between zebras (representative of domesticated equines) and wildebeests (representative of domesticated bovines), researchers found that zebras chose to graze the tallest and most fibrous part of the plant, the stem, which is generally comprised of less-soluble nutrients. The wildebeests chose the leafier, most nutrient-dense portions of the plant, and ate closer to the ground [46]. This is supportive of the thought that horses have evolved to consume large amounts of low quality forage throughout most of the day, especially when compared to other livestock species. This also supports the management practice of grazing animals, such as horses and cows, together to promote better pasture utilization and efficiency.

Studies have been conducted investigating the daily grazing behavior of feral horses, specifically determining the amount of time horses spend grazing each day. Fleurance et al. (2001) determined that horses spend about 75% and 50% of their time grazing during the day, and night, respectively [47]. This translates to a total of approximately 60% of each day consuming feedstuffs, or about 14.4 h. The rest of their time is usually comprised of standing, walking, lying, drinking and scratching. However, when the environment is altered and horses are not allowed to graze for long periods of time and are stalled or kept in confinement, stereotypies may develop. The most common stereotypies of confined horses are cribbing, stall walking or weaving, and wood chewing [48].

Horses are social animals and prefer to be kept in groups rather than individually housed. Houpt (1990) found that if two horses are grazing together, when one starts to eat, or stops eating, the other will follow. Several studies have found that horses who were simply allowed to see another horse, but not have physical contact with one another, had a decrease in the exhibition of stereotypies, further evidence to support the importance of group housing [39–42]. Group housing, combined with the importance of long periods of foraging, should be taken into consideration when devising an ideal horse management scheme.

Forage height and grass species offered tend to impact horse preference. Fleurance et al. (2010) found that the optimal grazing height of the grasses was 5 to 6 cm when horses were offered grasses of varying height. In a mixed grass height pasture, horses preferred grasses ≤8 cm. When pastures were taller, 9 to 40 cm, horses preferred grasses that averaged 22 cm. Additionally, horses chose to graze lawns instead of roughs (patches of grass with varying heights). Horses spent about 60% of their time grazing on short pasture compared to 55% and 51% of their time grazing mixed and tall, respectively, pasture [45].

Preferences for short grass heights could be advantageous for owners when grazing horses with a restrictive device such as a grazing muzzle. Lower amounts of forage should decrease the amount of plant biomass available for the horse to consume. However, nutrients are generally found in higher concentrations in shorter, less mature forages, and digestibilities are usually higher. Crude protein was found to be approximately 50% higher in shorter grass compared to taller grass, and taller grasses containing approximately 20% more NDF [44, 51]. Species grazed and stage of maturity should be taken into account when grazing with a restrictive device, providing another reason why investigating the interaction of grazing muzzles and various forage species is important to horse owners.

Equine forage intake estimation

Accurate estimation of pasture dry matter intakes (DMI) of grazing horses has proven problematic, as there are many influential factors. Some of the many factors include forage growth stage, forage species, palatability, forage height and individual horse behavior and grazing experience. Along with difficulties in measuring intakes, there are few estimates of pasture consumption rates of grazing horses over varying time periods of pasture access, making ration balancing for the grazing horse extremely difficult. Some methods that have been used by researchers include subtraction of residual herbage mass from initial herbage mass [52, 53], radioactive markers [54–56], fecal output measurements along with known organic matter and dry matter (DM) digestibilities [60], changes in BW and accounting for insensible weight loss and excretory outputs [61], or determination of bite size, number, and duration of feedings [62].

Rayburn et al. (1998) devised a method of herbage mass estimation using a plate meter to estimate sward height and density and resulting intakes from loss of herbage mass [63]. To accurately use the plate meter, multiple measurements were made in random order covering the entire pasture both before (initial) and after (residual) grazing. Sward height was measured at each harvest area and an estimation of herbage mass was made. The weight of the entire forage is then extrapolated to the entire area [63].

Estimates of pasture voluntary dry matter intakes (VDMI) range from 1.5- 3.1% BW [6]. Past studies have evaluated horses at different stages of growth, production and while grazing different types of pasture, making it difficult to extrapolate data to different management situations. Lactating mares usually consumed about 2.8% BW, while the remaining groups of horses consumed approximately 2% BW [6].

When evaluating pasture DMI, it is important to know the production stage of the horse as it affects intake estimates. Duren et al. (1989) evaluated DMI in yearling horses and the impact of exercise on intake, bite rate and bite size. Without exercise, yearlings ate at a rate of approximately 0.63 kg/h, while yearlings with exercise ate at a rate of approximately 1.75 kg/ h; however, no significant difference between the two treatments was observed [64]. Cantillon et al. (1986) estimated pasture DMI rates were between 1.5 and 1.65 kg/ h when adult horses grazed fescue and alfalfa pastures [65]. Duren (1987) estimated that exercised yearling horses had an intake rate of about 3.2 kg DM over a 3-hr grazing period, which he extrapolated to be a rate of about 1.07 kg DM/ h for a 24-h grazing period [66]. However, in both studies [49, 50], horses were tethered and not allowed free range of the pasture. Therefore, these results may not be representative of horses' natural pasture DMI rates. It has been theorized that tethered horses have a tendency to act tied instead of graze freely, decreasing the amount of time horse spend grazing [66].

Pasture nutritive value has also been shown to affect DMI in grazing horses. Nash et al. (2001) found that Thoroughbred fillies decreased DMI when grazing higher-quality pastures. These results conflict with others who have determined DMI increases on high-quality pastures [39,52]. This suggests that pasture DMI may not be entirely dependent on pasture quality, but rather on the amount of DE that the horses ingest from the plants, with some horses able to maintain a fairly homeostatic intake range of calories. However, with the recent increases in the number of obese horses, this would appear to be incorrect, with horses maintaining a positive energy balance for long periods of time.

Pasture DMI has been measured in other species, including cattle. Researchers found that cattle are able to consume extremely large quantities of forage in relatively short amounts of time. In one study, it was found that cattle were able to consume 90% of their daily needs in only 2 hours [69]. These results are similar to equine grazing intake rates when turnout was restricted. Dowler et al. (2009) evaluated pasture DMI in horses allowed to graze for 8 hours by comparing the first and second half (4 hours) of DMI while grazing. It was found that horses turned out for 8 h a day had a higher intake rate during the first 4 h compared to the second 4 h of grazing. In the first 4 h, the average DMIR was 2.2 g DM · kg BW^{-1} · h^{-1}, whereas in the second 4 h the intake rate decreased to about 0.9 g DM · kg BW^{-1} · h^{-1}, less than half of the first 4 h of grazing. Additionally, these horses had the ability to consume about 55% of their daily DE requirement in the first 4 h [56]. In a similar study, Glunk et al (2012) found that horses whose pasture access was restricted to 3, 6 or 9 h per day had much higher DMIR compared to horses who had continuous pasture access [55]. The horses had average pasture DMIR of 1.96, 1.5, 1.12, and 0.96 g DM · kg BW^{-1} · h^{-1} during 3, 6, 9 and 24 hour of grazing, respectively. Additionally, horses grazing for 3 h were able to consume approximately 55% of their daily caloric requirement, similar to the results found by Dowler et al. (2009) representing a large intake of calories in a short period of time.

The ability of horses to alter their DMIR makes accurately estimating pasture DMI difficult, especially when a horse's ration is largely based on pasture inputs. Additionally, when trying to decrease pasture DMI due to obesity or laminitis, it can be difficult to know the ideal length of grazing time necessary to achieve exercise and socialization while limiting caloric intake.

To better estimate pasture DMI based on length of time allowed to graze, the following equation was developed:

$$\text{Estimated amount of pasture consumed (g DM/ kg BW)} = 5.12\sqrt{x}-2.86$$

(where x is the number of hours of pasture access) [70]

Estimating the amount of pasture consumed will enable horse owners and professionals the ability to more accurately predict the amount of forage a horse is consuming. Combining results of this equation with forage nutritive values of the pasture grasses, providing both quantity and quality of nutrients a horse is consuming, enabling for more accurate ration formulation.

Horse intakes of preserved forage have also been evaluated. Martinson et al. (2011), found that when horses fed from round-bale feeders their daily DMI was greater compared to a no-feeder control [71]. The average DMI of horses feeding off the control was 1.3% BW, while average DMI of horses feeding from feeders was between 2.0 and 2.4% BW. Researchers concluded that the higher DMI was due mostly to a reduction in hay waste when a round-bale feeder was used. Hay waste from the no-feeder control was 57%, while waste resulting from the feeders ranged from 5 to 33%. A study by McMillan et al. (2010) found similar results. The use of a feeder significantly reduced waste, from 31% to 9% when feeding alfalfa round-bales and 38% to 2% when feeding coastal Bermuda grass round-bales. However, his study resulted in conflicting DMI when horses were fed different types of hay. While DMI were not significantly different between hay types, DMI were reduced from 10.46 kg DM without a feeder to 9.96 kg DM with a feeder when fed alfalfa, and increased from 7.53 kg DM without a feeder to 8.71 kg DM with a feeder when fed coastal Bermuda grass [72]. A third study had similar results, with hay waste ranging from 31% to 9% when horses were fed round-bales with a feeder [72].

Ability of forage to meet equine requirements

It is fairly easy for a horse to ingest excess nutrients from forage, especially fresh, nutrient-dense forage. Good quality pasture can supply all required daily nutrients for most horses, in exception to those with high metabolic requirements including lactating mares and performance horses [6]. Forages also play an essential role in maintenance of normal microbial function in the hindgut, which is needed for maximal fiber fermentation and volatile fatty acid (VFA) production.

Forage quality varies with forage species and maturity. For example, perennial ryegrass contains approximately 9 to 19% crude protein (CP) and 2.8 to 3.5 Mcal DE/kg, depending on maturity [76]. Reed canarygrass has similar amounts of CP, ranging from 12-18%, and NDF ranging from 54 to 63%, depending on variety, management system, and stage of maturity [73].

Grass species and maturity not only influence forage nutritive values, but horse preference. Several studies have been conducted looking at forage preferences of grazing horses. Watson (2008) offered horses a choice of six cool-season grasses, including Kentucky bluegrass (*Poa pratensis)*, orchardgrass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), timothy (*Phleum pretense*), festulolium and tall fescue (*Festuca arundiacea*). It was determined that horses preferred timothy and orchardgrass over the other species. Festulolium, perennial ryegrass, and annual ryegrass were intermediate choices of grazing horses. Tall fescue was least preferred. Watson (2008) showed that horses chose forages with higher levels of copper, zinc and potassium, and lower levels of magnesium. Crude protein, non-structural carbohydrate (NSC), water-soluble carbohydrate, ethanol-soluble carbohydrates, or starch content were not factors affecting horse preference [74].

Allen et al. (2012) also conducted a study investigating preferences of horses grazing cool-season grasses. They found that horses preferred timothy, Kentucky bluegrass and meadow fescue, and that horses did not prefer meadow bromegrass, creeping foxtail, reed canarygrass and orchardgrass. Average seasonal removals of the most preferred grasses were >60%, while removals of less preferred grasses were <50% [75].

A study conducted at the University of Kentucky found conflicting results with orchardgrass, and timothy, being the most preferred grass [76]. Festulolium and smooth bromegrass were intermediately preferred, and tall fescue and Kentucky bluegrass were least preferred. These studies have both similarities and differences, likely due to the fact that preference is a behavioral response of an animal to plants when a choice is given. Preferences of grazing horses are likely affected by location, plant maturity, environmental conditions, and other species present.

Once an accurate estimation of intake has been developed, it is then important to evaluate several nutrients within the horse's diet. These important nutrients include: digestible energy (DE), crude protein (CP), neutral-detergent (NDF) and acid-detergent fiber (ADF), and mineral content. Digestible energy is the amount of energy available to the horse for maintenance, growth, production, lactation and performance. Digestible energy is obtained by subtracting the amount of energy that is undigested and lost in the feces from the gross energy (GE). Pagan (2005) also developed the following DE estimation equation based on the nutrients found within the plant.

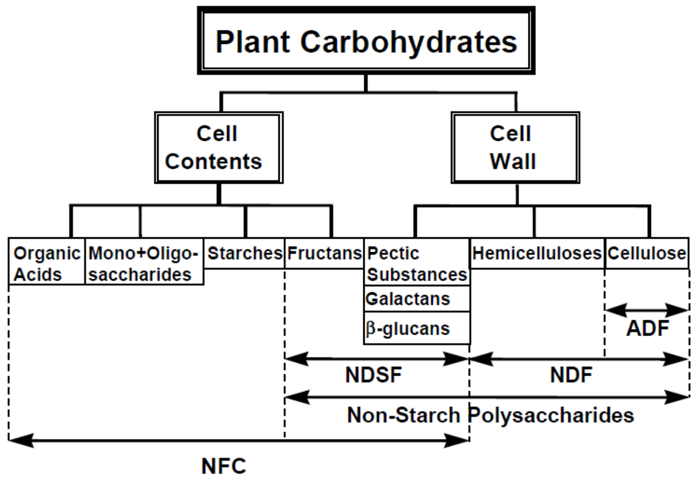
Digestible energy (Mcal/ kg DM) = 2.118 + 0.01218 CP - 0.00937 ADF - 0.00383 (NDF - ADF) + 0.04718 EE + 0.02035 NFC - 0.0262 Ash [77]

where EE= ether extract and NFC= non-fiber carbohydrate

The DE equation accounts for the input of calories from various chemical sources of the plant, including CP, hemicellulose, fat, and non-fiber carbohydrates (NFC), while subtracting the amount of ash or minerals that is in the plant. Crude protein, ADF and NFC are digestible or fermentable and increase DE, compared to NDF and ash which lower DE due to low digestibility [78].

Non-fiber carbohydrates are often used interchangeably with NSC, which is not correct. While both NFC and NSC are important sources of energy in a horse's diet, NSC's refer to the sugar, starch and other cell contents of the plant, while NFC is a calculated value. NFC is estimated using the equation: NFC= 100- %NDF- % Fat- % Ash- % CP [79]. The four general categories of NFC are organic acids, mono- and oligosaccharides, starch, and NDF. Although organic acids are not true carbohydrates, they are plant organic acids that are found in fresh forage, hay, and silage. The predominant sugars that are found in plant sources are glucose, fructose and sucrose. Most sugars are able to be digested by mammalian enzymes, and can be absorbed by the horse prior to fermentation. Starch is able to be digested by both mammalian and microbial enzymes, and can be absorbed prior to fermentation in the hind gut, depending on the processing, storage method and source of the starch. The final category, NDF, contains pectins, B-glucans, fructans, and other non-starch polysaccharides. Sources of NDF are unable to be digested by mammalian enzymes, and therefore must be fermented by the microbes in the hindgut of the horse [81; Figure 1]

Figure 1. Schematic of plant carbohydrate constituents and their portion of carbohydrate chemical analysis.



Excess intakes and metabolic effects

While ingesting adequate amounts of forage is crucial to the health of the equine gastrointestinal tract, problems can result when excess DE intakes occur that can have detrimental impacts on overall horse health. Excess intakes, if continued for a prolonged period of time, can lead to obesity, development of equine metabolic syndrome, exercise intolerance, insulin resistance, and laminitis [4,7, 8, 28, 59].

Excess energy intakes most often leads to accumulation of fat depots throughout the body. Most notably, this occurs on the buttocks, abdomen and neck, although this can vary by breed [81]. Several studies investigating the prevalence of obesity in horses have found that the condition is severely under recognized, and some owners actually prefer their horses to be maintained at a higher bodyweight. In one study, it was found that 45% of 319 horses evaluated were categorized as “fat” or “very fat” (5 or 6 on a 6 point scale) [82]. A similar study found approximately 32% of 300 randomly sampled horses were categorized as over conditioned (body condition score (BSC) of 6.5 to 7), while approximately 19% were obese (BCS 7.5 to 9) [82], exceeding the prevalence of obesity in household pets including cats (26% obese) and dogs (25%) [83].

Obese horses often have a decreased number of insulin receptors [84] or altered intracellular insulin signaling [85]. This decreases glucose’s ability to enter the cell, leading to maintained elevated blood glucose and insulin levels, meaning the horse has become insulin resistant. The liver, in response to the high levels of insulin, takes up glucose and converts it to fat for storage, resulting in an increase in adipose tissue and obesity. This also leads to pancreatic exhaustion and decreased insulin production, ultimately resulting in insulin resistance [76, 77]. Maintaining proper forage (and nutrient) intakes are key to horse health 0and maintenance of optimal gut health.

Additionally, obesity has been linked with laminitis [63, 64, 65, 66], pituitary pars intermedia dysfunction (PPID) [82, 68], osteochondrosis [94], hyperlipemia [95], diabetes mellitus [93] and endotoxemia [96]. Chronic insulin resistance in obese horses has been suggested to be the cause of PPID, especially in horses that have been fed energy-dense rations for extended periods of time [97]. Pathogenesis of hyperlipemia syndrome starts with excessive mobilization of lipid stores from excess adipose tissue reserves in obese horses when the horses are in negative energy balance. This excess circulating plasma triglyceride concentrations leads to circulatory disturbances and organ failures due to fatty infiltration [98]. Obesity has also been associated with horses with chronic laminitis, and elevated levels of inflammation from corticosteroids [97, 9]. Abdominal adipose tissue also has increased expression of the steroid converting enzyme 11-hydroxysteroid dehydrogenase-1 (11-HSD1). This enzyme increases the local activity of cortisol and is found in high concentrations in subcutaneous adipose tissues [97]. The presence of elevated concentrations of 11-HSD1 could result in further increased cortisol concentrations, further increasing the likelihood of obesity and laminitis.

Fat, or adipose tissue, in obese horses regenerates cortisol, a glucocorticoid that is produced in the adrenal cortex. Normally, cortisol is released in response to stress and is responsible for increasing blood glucose, suppressing the immune system, and aiding in fat, protein and carbohydrate metabolism [106]. Cortisol secretion, has also been shown to be linked to adiposity [106]. Cortisol is an important metabolite in the etiology of equine metabolic syndrome, causing increased accumulation of adipose, and decreased insulin production [86]. It has been debated whether horses exhibit a circadian rhythym, similar to what is found in humans, monkeys, and rats [107]. Several studies in which horses were housed and fed in a fairly controlled environment found that these horses exhibited a circadian cortisol secretion pattern, with peaks around 0600 and 0900, and troughs between 1900 and 2300 [96–99]. However, other studies found that these patterns do not or only occasionally occur in horses [100–103]. It may have been that the stress of the research tests masked the normal daily cortisol secretion patterns, or that a consistent, daily routine may have created an artificial circadian rhythym. A subsequent study found that horses sampled in their home paddock had a distinct circadian cortisol rhythm, while horses placed in a different environment had no circadian rhythm [107]. This was due to the concentrations during the normal circadian trough being raised, while peak cortisol concentrations were unaffected.

Cortisol can have a large impact on glucose secretion, serving as a stimulant of gluconeogenesis as well as mobilization of amino acids and fatty acids [116]. While beneficial to the horse under stressful situations, it can be detrimental to horses with metabolic syndrome, continuing the cycle of increased levels of adiposity and elevated glucose levels.

Current methods to decrease pasture intake

There are several methods implemented by horse owners and professionals to decrease nutrient intake, specifically DE and NSC. Two of the common methods for restricting pasture access are utilization of a drylot and grazing muzzling. The use of a grazing muzzles allows horses to socialize and exercise while decreasing forage intake [61]. Longland et al. (2011) investigated differences in pasture intakes over a three hour period, comparing ponies' DMI with and without grazing muzzles. Forage intakes were measured by calculating the ponies' insensible weight loss (ISWL) and excretory outputs, subtracting both from live body weight. Results showed that pasture intakes were significantly reduced when the ponies were fitted with a grazing muzzle. Pasture DMI of the muzzled ponies averaged 0.14% live body weight in three hours, compared to an estimated intake of 0.8% of live body weight in three hours without the grazing muzzles, resulting in a decreased intake of 83% when grazing muzzles were used. Also highlighted in these findings were that un-muzzled ponies were able to consume about one half to two-thirds of their daily total DMI in three hours, leading the potential to underestimate pasture intakes of ponies, similar to results observed with horses [54, 55, 82]. Additionally, as these ponies became accustomed to the amount of time they were on pasture, their DMIR were altered. Ponies at the end of the 6 week study had the ability to adapt, and had higher intake rates compared to the intake rates at the onset of the study. Further investigation into pasture intakes and utilization of grazing muzzles is warranted, as the methodology used in the previous study (ISWL) may not be effective in accurately estimating pasture intakes and it is important to know how well horses and ponies have the ability to adapt to restricted turnout.

Another method employed by horse owners and managers to reduce pasture intake is to restrict or eliminate a horses' access to pasture (often to less than a few hours a day) and feed them two to three large, concentrate-based meals [118]. While this allows for accurate ration balancing when done by a knowledgeable manager, it can be detrimental to horses' physical and mental health. Often, horses that are kept in confinement for a majority of the day develop stereotypies such as stall weaving or walking, cribbing, windsucking and wood chewing [48]. These stereotypies are used as a coping mechanism due to decreased social interaction and physical activity, and are usually increased or intensified around meal times. These horses also tend to have decreased meal consumption times which can lead to other adverse health effects such as ulcers and laminitis, often due to decreased gastric and colonic pH [11]. For these horses, it is recommended to increase their total time to consumption to potentially decrease the exhibition of stereotypies and the risk of negative health effects.

CHAPTER II: THE INTERACTION OF GRAZING MUZZLE USE AND GRASS SPECIES ON FORAGE INTAKE OF HORSES

1.0 Introduction

Recent research has shown that 21, 19, and 14% of horses in the United Kingdom [2], New York [3], and Minnesota [4], respectively, were considered "fleshy" with a body condition score [81] of ≥ 7. In an attempt to reduce horse body condition, owners have sought to restrict forage intake by a number of methods, including eliminating or decreasing the amount of time on pasture, however; restricted grazing is not always effective. Glunk et al. [119] found that horses were able to increase their dry matter intake rates with restricted grazing time.

Many horse owners are in need of management strategies that restrict pasture intake while maintaining a horse's natural environment. In recent years, the use of grazing muzzles has gained popularity because its use limits forage intake while still allowing turn-out, exercise, and socialization in an outdoor setting. Longland et al. [61] found that utilization of a grazing muzzle reduced forage intake by 83% when ponies grazed an autumn pasture with a sward height of 8 to 15 cm. However, horses are known to be selective grazers, which may affect the effectiveness of a grazing muzzle. Allen et al. [75] determined that horses preferred Kentucky bluegrass (*Poa pratensis* L.) and meadow fescue (*Schedonorus pratensis* Huds.), while exhibiting less preference for reed canarygrass (*Phalaris arundinacea* L.) and perennial ryegrass (*Lolium perenne* L.). Researchers have observed that grass morphology, or growth type, also affected livestock forage preference [120]; however, it is unknown if horse preference and forage morphology will impact the effectiveness of a grazing muzzle. Therefore, the objective of this research was to determine the effectiveness of grazing muzzle use at reducing forage intake when horses grazed grasses with different morphology and preference.

2.0 Materials and Methods

2.1 Horses, Forage, and Sampling

All experimental procedures were conducted according to those approved by the University of Minnesota Committee on Animal Use and Care. On August 8, 2011, six replicated plots measuring 3.3 x 6.7 m were planted. Grass species included ‘Ginger’ Kentucky bluegrass, ‘Remington’ perennial ryegrass, ‘Pradel’ meadow fescue and ‘Palaton’ reed canarygrass. Kentucky bluegrass and meadow fescue were previously determined to be highly preferred, while perennial ryegrass and reed canarygrass were determined to be less preferred [75]. Kentucky bluegrass and perennial ryegrass have a prostrate growth habit, while meadow fescue and reed canarygrass have an upright growth habit. Therefore, horses were exposed to grasses that were preferred with prostrate growth (Kentucky bluegrass), preferred with upright growth (meadow fescue), less preferred with prostrate growth (perennial ryegrass) and less preferred with upright growth (reed canarygrass).

Research was conducted in June and August of 2012, and August and September of 2013 when grasses reached a height of 15 to 20 cm [75,121]. In 2012 all four grass species were grazed, while in 2013, only Kentucky bluegrass and reed canarygrass were grazed due to winterkill of perennial ryegrass and meadow fescue.   In 2012, a Latin square design utilized four of the six replicates, while in 2013 a Latin Square design was used with six replicates. Prior to grazing in 2012 and 2013, four adult stock-type horses with a body weight (BW) of 406 kg (SD ± 107 kg), and three adult stock-type horses with a BW of 557 kg (SD ± 34 kg), respectively, were acclimated to both wearing a grazing muzzle (Weaver, Mt. Hope, OH) and grazing for 4 hours each day on a mixed, cool-season grass pasture for one week. . Prior to grazing, horses were weighed on a livestock scale and initial herbage mass was measured by mechanically harvesting a 0.9 x 3.3 m strip from each plot using a flail harvester (Carter Manufacturing Company, Inc., Brookston, IN) at approximately 0800 hours. Harvested samples were weighed, and subsamples were collected and dried at 60°C in an oven for 24 hours to determine dry matter (DM).

In 2012, horses were allowed access to two of the four grasses each month. Horses were allowed to graze each species for two consecutive days, one day with the muzzle and one day without the grazing muzzle on a different plot containing the same species. Horses were then switched to the second grass species and the protocol was repeated. The following month the protocol was repeated to ensure each horse had access to each grass species both with and without a grazing muzzle. In 2013, horses grazed for four consecutive days each month, with access to both species of forage each month, both with and without the grazing muzzle. While grazing, horses had *ad libitum* access to water.

After each grazing period was completed, residual herbage mass was estimated by mechanically harvesting a second 0.9 x 3.3 m strip from the opposite side of each plot using the flail harvester at approximately 1300 hours. Harvested samples were then weighed, and subsamples were collected and dried at 60°C in an oven for 24 hours to determine DM. Manure was removed, and each plot was mowed to 9 cm and allowed to re-grow [75,121]. To estimate total herbage mass consumed, herbage mass densities were calculated from the initial and residual herbage masses using the following equation:

Density (kg/m^2) = weight of strip harvested (kg)/ area of strip harvested (m^2)

The density was then extrapolated to the entire plot and the difference between initial and residual herbage mass was determined to be the amount of forage consumed by the horse.

During the trial period in both years, horses were group housed in a nearby dry lot with *ad libitum* access to water and a trace mineralized salt block, and were group fed a mixed, mostly cool-season grass hay at 1% of herd BW at 1600 hour each day. When not grazing, horses were housed in the same dry lot with *ad libitum* access to water and a trace mineralized salt block and were group fed a mixed, mostly cool-season grass hay at 2% of herd BW split evenly at 0700 and 1600 hours each day. Rations were balanced to meet the horse's nutritional requirements during and between grazing periods [122].

2.2 Statistical Analysis

Data were analyzed using the Proc Mixed procedure of SAS (9.3, SAS Institute, Cary, NC). Variables analyzed included percent initial herbage mass consumed and percent reduction. The model included period, species, and muzzle. A $P < 0.05$ was considered statistically significant.

3.0 Results

Average percent initial herbage mass consumed and percent reduction in year 1 (2012) is shown in Table 1. There was no effect of species ($P = 0.27$) on initial herbage mass consumed. Although a wide range in consumption values were observed both with (22 to 49%) and without (47 to 79%) a grazing muzzle, consumption was not different among the forage species. This was likely due to natural variability in forage height and density found within the plots. However, average initial herbage mass consumed was reduced by 29% when the horses grazed while wearing a grazing muzzle, representing a reduction ($P \leq 0.05$) in consumption for all species except reed canarygrass. Average percent initial herbage mass consumed and percent reduction in year 2 (2013) are shown in Table 2. Similar to the previous year, the use of a grazing muzzle was effective at decreasing initial herbage mass consumed by an average of 30% ($P < 0.001$). However, unlike 2012, there was an effect of species ($P = 0.042$) on percent initial herbage mass consumed. Horses consumed more Kentucky bluegrass compared to reed canarygrass both with and without the use of a grazing muzzle.

4.0 Discussion

The use of a grazing muzzle decreased the percent of initial herbage mass consumed across all species, except reed canarygrass in year 1. Allen et al. [75] found that reed canarygrass was less preferred by grazing horses compared to other cool-season grasses which likely contributed to the similar initial herbage mass consumed both with and without the use of a grazing muzzle in year 1. Another possible contributing factor was the morphology of reed canarygrass. Reed canarygrass has an upright and stiff leaf and stem, possibly making it easier for the horses to consume while wearing the muzzle. However, in year 2, there was a reduction in initial herbage mass consumed when horses grazed reed canarygrass with the muzzle. It is possible the horses began to acclimate to grazing reed canarygrass in year 2, helping to explain the difference between the two years. Other researchers have determined that horses can acclimate to feeding systems over time [119,122].

Previous research [75] determined that horses preferred Kentucky bluegrass. However, in the current trial, the only difference in initial herbage mass consumed was observed in year 2 between Kentucky bluegrass and reed canarygrass. These results provide further evidence that horses prefer Kentucky bluegrass over reed canarygrass. It is unclear why differences in initial herbage mass consumed were not observed during year 1. However, the relatively short grazing period and natural variability in forage height and density found within the plots may have contributed to the inability to detect differences. Future research should investigate the effect of grazing muzzle use on forage intake over longer time periods.

The average 30% reduction in herbage mass while wearing a grazing muzzle observed in the current study is much less than the 83% reduction previously reported by Longland et al. [61]. However, Longland et al. [61] began grazing ponies on an autumn pasture when sward heights reached 8 to 15 cm. In the current study, horses began grazing the plots when forage heights reached 15 to 20 cm, a common recommendation for cool-season grass pastures [75,121]. It is possible the height of the sward at the time of grazing impacted the effectiveness of the grazing muzzle. Future research should focus on the effect of sward height on grazing muzzle effectiveness. Along with sward height, the use of ponies, grazing a pasture of unknown species and composition, and the use of insensible bodyweight loss to estimate forage intake are likely the major factors contributing to the different results between the two studies.

The use of barriers has also been used to reduce or slow horse intake while feeding hay and grain. Glunk et al. [123] determined that using a slow feed hay net (3.2 cm openings) reduced intake rates compared to feeding hay off the stall floor. When feeding grain, Kutzner-Mulligan et al. [124] determined that feed consumption time was greater when either balls or a waffle obstacle was added to a bucket compared to a control. These data agree with the current results that barriers or obstacles can be used to reduce or slow consumption of hay, grain and pasture when feeding horses.

5.0 Conclusions

The use of a grazing muzzle reduced adult horse's pasture intake by approximately 30%, regardless of cool-season grass species grazed. Use of a grazing muzzle appears to offer a simple, affordable, and effective management strategy for restricting forage intakes of grazing horses. Results will be useful in helping horse owners, veterinarians, and nutritionists estimate forage intake of muzzled horses on pasture and will be useful in developing more accurate rations for muzzled horses.

CHAPTER III: THE EFFECT OF HAY NET DESIGN ON RATE OF FORAGE CONSUMPTION AND HAY WASTE WHEN FEEDING ADULT HORSES

1.0 Introduction

Equines have evolved as hindgut fermenters, physiologically designed to consume frequent, small forage-based meals throughout the day [1]. Fleurance et al. [53] estimated that horses in a natural setting spent about 14 hours grazing each day. However, modern management systems tend to limit a horse's opportunity to forage to approximately 9 hours each day [48]. Many performance horses are stalled, fed large amounts of concentrated grain meals, and have feedings limited to two or three times daily [48]. This common management scenario can result in deleterious health and behavioral issues, including development of ulcers [14], an increased risk of colic [37,44], and behavioral vices including wood chewing, crib-biting, and stall walking [125]. Access to long periods of foraging tends to decrease deleterious health issues and some behavioral vices [37,44,125,126]. Furthermore, many horse farms tend to have high stocking rates and inadequate amounts of pasture further limiting foraging opportunities [121]. Because of this, many horse owners and managers struggle to replicate the amount of time horses spend foraging in a natural setting.

Slow feed hay nets are newly developed products being marketed to horse owners, managers and professionals as a way of replicating the natural foraging behavior of horses in modern horse management systems. Although slowing hay consumption has not been investigated, researchers have been successful at increasing time to consumption of grain with the use of obstacles in a feed bucket [124,127]. Consumption rates of horses feeding from slow-feed hay nets is unknown, but presents a possible management strategy for increasing foraging time in modern horse management systems. The objectives of this research were to determine the effect of hay net design on rate of forage consumption when feeding adult horses. It was hypothesized that as hay net opening size decreased, time to consumption would increase and forage intake rates would decrease.

2.0 Materials and Methods

2.1 Study 1

2.1.1. Animals and Treatments

All experimental procedures were conducted according to those approved by the University of Minnesota Committee on Animal Use and Care. Eight adult stock-type horses in light work, with an average body weight (BW) of 513 kg (SD ± 47 kg) were used in a replicated Latin Square design, with 2 horses per treatment per week. Horses were fed in individual stalls (3.0 x 3.7 m) either off of the floor (control), or from one of three hay nets: large (15.2 cm openings), medium (4.4 cm openings), and small (3.2 cm openings). The medium and small hay nets were manufactured by Cinch Chix LLC (North Branch, MN), while the large hay net was manufactured by Weaver (Weaver, OH). Hay nets were made of webbed fabric with diamond shaped openings. The medium and small hay nets were made of UV-treated Dupont® fiber while the large hay net was made of nylon roping. Horses were acclimated to treatments for 2 days, followed by 3 days of data collection, and then a 2 day wash-out period. Every 7 days, horses were reassigned to a different treatment. The protocol was followed for 8 weeks, during which each horse ate from each treatment twice (n = 16). Period 1 included weeks 1 through 4, while period 2 included weeks 4 through 8. Mixed, mostly grass hay was fed at 1.0% BW twice each day at 0700 and 1600 hours, for a total of 2.0% BW daily [6]. During each feeding, hay was available to the horses for 4 hours. Prior to feeding, hay was weighed and placed in the hay net or on the stall floor. Amount of hay offered to each horse was dependent on BW and ranged from 4.3 to 5.7 kg per feeding. After the 4 hours had expired, hay nets were removed and any hay remaining in the hay nets was weighed and considered an ort.

When not stalled, horses were ridden in a university introductory riding class and housed in an outdoor dirt paddock. No additional hay was fed while housed in the paddock. During the 2 day wash-out period, horses were group housed in the paddock and fed the same hay at 2.0% of the total herd BW per day split evenly into meals at 0700 and 1600 hours. Horses had *ad libitum* access to water when housed in the stalls and paddock, and had *ad libitum* access to a trace mineralized salt block while in the paddock.

2.1.2 Sample Collection and Analysis

Each week, multiple small-square bales of hay were cored (2 x 51 cm) to determine forage nutritive value. Weekly samples were combined (n=8) and analyzed for forage nutritive value by a commercial forage testing laboratory (Equi-Analytical, Ithaca, NY) using the following methods. Dry matter (DM) was determined by placing samples in a 60°C forced air oven for 24 hours (method 991.01) [128]. Crude protein was calculated as the percentage of N multiplied by 6.25 (method 990.03) [128]. Neutral and acid detergent fibers were measured using filter bag techniques [129–131]. Starch and water and ethanol soluble carbohydrates were measured using techniques described by Hall et al. [79]. Mineral concentrations were determined (Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma Radial Spectrometer, Thermo Instrument Systems Inc., Waltham, MA) after microwave digestion (Microwave Accelerated Reaction System, CEM, Mathews, NC). Equine DE was calculated using an equation developed by Pagan [132].

During the 8 week study period, edible refuse hay on the stall floor that remained after the 4 hour time period was collected, dried at 60°C in an oven, weighed, and subtracted from the amount fed. Edible refuse hay was not contaminated with urine or feces. Percent of hay consumed was determined (on a DM basis) using the following equation:

Percent consumed = (total amount offered – ort – edible refuse hay / total amount offered) x 100

No bedding was used during the study to aid in the collection of edible refuse hay.

After observing a visual difference in hay waste among the treatments during period 1, hay waste was collected during period 2. Hay waste was hay contaminated with feces or urine. Hay waste was hand separated from manure and urine, dried at 60°C in an oven, weighed, and percent hay waste was calculated (on a DM basis) using the following equation:

Percent hay waste = (amount of hay waste/ amount of hay offered) x 100

Time to consumption was determined using a stopwatch. Stopwatches were started immediately after horses began eating and were stopped once horses either finished all offered hay or the 4 hour time period had expired. If during the 4 hour time period horses did not show an interest in eating for ≥ 10 minutes, the stopwatches were stopped. However, if horses began eating again within the 4 hour time period, the stopwatches were started once again. This process was repeated until either all hay was consumed or the 4 hour time period had expired.

2.1.3. Statistical Analysis

All data was analyzed using the Proc Mixed procedure of SAS 9.3 (SAS Institute, Cary, NC). Variables analyzed were percent consumed, time to consumption (hours) and dry matter intake rate (DMIR, kg DM/ hour). The model included period, treatment, square, week, horse and treatment x week. There was no effect of horse ($P > 0.05$); therefore, the mean values from horses within a treatment were analyzed. Results were considered significant at $P < 0.05$ and are expressed as Least Squares Means (± SE).

2.2 Study 2

2.2.1 Animals and Treatments

Because none of the horses feeding from the small hay net, and only 5 of the 8 horses feeding from the medium net in study 1 were unable to consume their hay meal in 4 hours, a second study was designed to determine the time to consumption when adult horses were fed from the medium and small hay nets. The same eight horses, hay, and management system was used in a second study; however, only the medium and small hay nets were evaluated using a crossover design. Four horses were assigned to either treatment for a period of five consecutive days, with a two day acclimation period preceding data collection. At the end of 7 days, the horses switched treatments and the process was repeated. Horses were fed once daily at approximately 0700 h and were allowed continuous access to the hay until they either finished all offered hay or no longer showed interest in eating for ≥ 10 minutes.   After horses had consumed the morning hay meal, they were group housed in an outdoor, dirt paddock and fed the same hay at 1.0% of the total herd bodyweight at 1600 hours.

2.2.2. Statistical Analysis

All data was analyzed using the Proc Mixed procedure of SAS (Cary, NC). The dependent variables analyzed were time to consumption (hours) and DMIR (kg/h). The model included treatment, period and sequence. Results were considered significant at $P < 0.05$ and are expressed as Least Squares Means (± SE).

3.0 Results

3.1 Hay Nutritive Value

Forage nutritive values for the mixed, mostly grass hay are listed in Table 1. When compared to a national hay nutritive value database [133], the hay was within or near normal ranges for all nutrients tested for mixed, mostly grass hay.

3.2 Study 1

Mean percent of hay consumed during the 4 hour period was 95, 95, 89, and 72% (SD ± 1.6) for the control, large, medium, and small hay nets, respectively. There was no difference between percent of hay consumed for horses feeding from the control and large hay net ($P = 0.939$). However, horses feeding from the medium hay net consumed a smaller percentage of hay ($P = 0.03$) compared to horses feeding from the large hay net and control, and horses feeding from the small hay net consumed the least percentage of hay compared to other treatments ($P < 0.0001$).

Mean time to consumption was different between the control and large hay net ($P < 0.0001$; Figure 1). Mean time to consumption was 3.1 and 3.4 hours for the control and large hay net, respectively, with horses feeding from the control requiring less time to consume their meal compared to the large hay net. Most horses feeding from the medium and small hay nets were not able to consume all forage during the 4 hour time period. Time to consumption was not affected by period ($P = 0.6271$).

Over the 4 hour feeding period, DMIR (kg/hour) were different among all treatments and decreased as hay net opening size decreased ($P < 0.0001$; Figure 2). Dry matter intake rates were 1.5, 1.3, 1.1 and 0.9 kg DM/ hour for horses feeding from the control, large, medium, and small hay nets, respectively. Although there was no difference between the percentage of hay consumed between the control and large hay net, there was difference in the time to consumption, which contributed to the difference in DMIR. The large hay net did not affect the horses' ability to retrieve and consume forage, but did slow the rate of feeding compared to the control.

Mean hay net orts after 4 hours of feeding were 0.08, 0.42, and 1.71 kg DM (SD ± .051) for the large, medium, and small hay nets, respectively, with all treatments being different from one another ($P < 0.05$). This provides additional evidence that the medium and small hay nets reduced the horses' ability to retrieve and consume forage compared to the large hay net and one another.

Mean hay waste after 4 hours was similar (*P* = 0.107) among all treatments. Hay waste was 0.12, 0.07, 0.08, and 0.03 kg DM for the control, large, medium, and small hay nets, respectively.

Equine DE intake during the 4 hour feeding period was different among most treatments (*P* <0.0001). Horses feeding from the small hay net consumed less calories (7.2 Mcals) compared to horses feeding from the medium hay net (9.2 Mcals). Horses feeding from the control and large hay net consumed the greatest amount of calories at 9.8 and 9.9 Mcals, respectively, and were not different from one another (*P* = 0.6271). However, if given additional time to consume the hay meal, it is likely all horses would have consumed a similar amount of calories per feeding.

3.2 Study 2

Mean time to consumption was different between the medium and small hay nets when horses were given an unlimited amount of time to consume the hay meal (*P* < 0.0001). Mean time to consumption for the medium and small hay nets were 5.1 and 6.5 hours, respectively (Figure 1). There was a decrease in DMIR when horses were fed from the small hay net compared to the medium hay net (*P* < 0.0001; Figure 2). Mean DMIR were 0.99 and 0.72 kg DM/ hour for the medium and small hay net, respectively, agreeing with the results from study 1 that determined the medium and small hay nets extended foraging time and decreased rate of forage consumption.

4.0 Discussion

The results support the hypothesis that decreasing hay net opening size limited horse access to hay and resulted in increased time to forage consumption and decreased forage intake rates in adult horses. Horses feeding from the medium and small hay nets took longer to consume their hay meal resulting in a reduced dry matter intake rate compared to horses feeding from the control and large hay net. Although determining the rate of hay consumption when feeding from a hay net had not been previously evaluated, other researchers have documented that barriers can slow pasture and grain consumption. Longland et al. [122] determined that using a grazing muzzle reduced pasture intake by 83%, while Glunk et al. [134] determined that using a grazing muzzle reduced pasture intake by an average of 29%. The use of obstacles in a feed bucket increased time to feed consumption by 20 to 80% [124,127]. These results show that horse owners and professionals can utilize various methods to slow consumption of hay, pasture and grain when feeding adult horses.

While horses feeding from the control and large hay net were able to finish their hay meal within the 4 hour period, horses feeding from the medium and small hay net were not. Previous research has shown that horses can acclimate and adapt to different amounts of feeding time. Longland et al. [122] found that ponies adapted to reduced grazing periods over 6 weeks and consumed forage more quickly when time on pasture was restricted. Glunk et al. [119] and Dowler et al. [117] also found that limiting grazing time led to increased DMIR compared to horses that were allowed to graze continuously. Long term studies feeding horses from the medium and small hay nets are warranted to determine if horses will adapt to feeding from the hay nets and alter DMIR over time.

Results from study 2 showed that horses required 5.1 and 6.5 hours to consume their morning hay meal when fed from the medium and small hay net, respectively. In comparison, horses fed from the control consumed their hay meal in 3.1 hours. In a natural setting, horses spend about 14 hours foraging each day [47], compared to modern management systems where a horse's ability to forage has been reduced about 9 hours each day [48]. Using the data from study 2, if a horse is fed 2% BW [6] each day split evenly between two meals and fed from the small hay net, foraging time would be approximately 13 hours daily, similar to the foraging time observed in a natural setting. Conversely, horses fed the same amount of hay from the stall floor (control) would spend about 6 hours a day foraging, leading to a greater than 50% reduction in the amount of time spent foraging compared to horses feeding from the small hay net. Research investigating daily time to consumption when horses are fed solely from medium and small hay nets is warranted.

There are likely several benefits to increasing time to consumption while simultaneously reducing forage intake rates for many adult horses. Increasing feeding frequency can lead to cycles of accelerated hindgut fermentation, mostly due to increasing rates of feedstuffs passage through the digestive tract [55]. Increasing foraging time also increases digestibility of feedstuffs by decreasing the amount of feed introduced into the digestive tract at one time [135]. There may also be health incentives to feeding forage from medium and small hay nets. Extending foraging time has been recommended as a strategy for reducing the incidence and severity of some behavioral vices, including crib-biting and wind-sucking [125,135]. A study investigating the risk factors of colic in horses found that feeding hay on the ground increased risk of colic versus feeding hay from a feeder [45]. Medium and small hay nets also provide a physical barrier restricting the horse from burying its muzzle into the hay. This is a potential benefit for horses diagnosed with respiratory diseases [136]. The physical barrier may also decrease the chance of bolting large quantities of forage which can lead to choke [137]. The effect of medium and small hay nets on horse health and behavioral vice should be further investigated.

However, the medium and small hay nets may not be ideal for all horses. Horses requiring large quantities of feed over a short period of time should not be fed from the medium or small hay nets. Also, horses with little incentive to eat may be discouraged from feeding from the medium and small hay nets. Observations from the current study included some frustration (i.e. biting and shaking the hay net) from horses during the trial, most often occurring during the first 4 feedings horses were fed from the medium and small hay nets. The medium and small hay net manufacturer does recommend acclimating naïve horses to feeding from the medium and small hay nets by first staring with a large hay net, followed by the medium hay net, and ending with the small hay net over the course of about a week.

Although hay waste was not different among treatments in the current study, Martinson et al. [71] found that using a hay net when feeding round-bales resulted in 6% hay waste compared to 57% waste when no round-bale feeder was used. Other researchers have also determined that using feeders reduced hay waste when feeding small square-bales in a stall [138] and when feeding round-bales [72]. Using a feeder confines hay and prevents horses from dispersing hay throughout the environment, reducing the chance of contamination by trampling, feces and urination, and therefore reducing the amount of hay waste. Furthermore, Carter et al. [127] determined that grain waste was reduced when horses were fed from a bucket that included an obstacle. The controlled feeding amount and prompt clean-up after 4 hours provide possible explanations as to why a difference in hay waste was not observed in the current study.

5.0 Conclusions

The use of hay nets with medium and small openings offers horse owners and professionals a practical and affordable option to reduce dry matter intake rates and extend foraging time in adult horses. Future research utilizing medium and small hay nets should focus on a horse's ability to adapt to feeding from the hay nets over time, daily time to consumption when fed solely from the hay nets, and the effect of hay nets on horse health and behavioral vices.

CHAPTER IV: THE EFFECT OF SLOW-FEED HAY NETS ON BODY WEIGHT AND POST-PRANDIAL METABOLITE PATTERNS IN HORSES

1.0 Introduction

Horses are hind-gut fermenters and have evolved to consume small, frequent forage meals throughout the day [1]. However, modern management strategies including meal feeding and increased length of stalling have led to the decreased ability of horses to forage [48]. This change in foraging behavior has led to alterations in hindgut fermentation and metabolic hormonal patterns, contributing to colic, laminitis, equine metabolic syndrome, insulin resistance, and obesity [85,86,139,140].

Post-feeding measurement of glucose, insulin and cortisol are important to determine the impact of a meal on a horse's digestive system. Changes in blood glucose and insulin are helpful in estimating the digestibility and absorption of a meal, with. elevated post-feeding values often correlated with diets that produce a more negative effect in the hindgut [141,142]. Stull and Rodiek (1988) found that different diets did not affect cortisol levels, but stress has been shown to impact cortisol levels [143]. In an effort to decrease the metabolic postprandial response to meal feeding, researchers have attempted to increase feedstuff total time of consumption by decreasing intake rate [123] and rate of passage through the digestive tract [124]. The objectives of this study were to determine if restrictive feeding combined with the use of a slow-feed hay net would affect weight loss, morphometric measurements, and postprandial metabolite patterns in overweight adult horses.

2.0 Materials and Methods

2.1 Animals, Management, and Experimental Design

All experimental procedures were conducted according to those approved by the University of Minnesota Committee on Animal Use and Care. Eight adult Quarter Horses (5 mares and 3 geldings) with a BW of 563 kg (SE ± 4.6 kg) and BCS [81] of 7.2 (SE ± 0.3) were used in a completely randomized block design for a period of 28 d. Horses had no known metabolic conditions, other than being overweight. Upon arrival, all horses were quarantined for 3 d followed by a 7 d acclimation period in individual boxstalls (3.0 x 3.7 m) at the University of Minnesota Large Animal Hospital. During this time, horses were fed grass hay at 2.5% BW split evenly between two meals at 0700 and 1600 h. After the quarantine and acclimation period, horses were blocked by bodyweight, BCS, and gender, and assigned to one of two treatments for a 28 d period. Treatments consisted of 4 horses consuming hay off of the stall floor (FLOOR), while the remaining 4 horses consumed hay from a slow-feed hay net (NET). The slow-feed hay net was made from webbed UV-treated Dupont® fiber with 3.2 cm diamond shaped openings (Chinch Chix LLC, North Branch, MN).

On day 1 of the data collection period, horses were fed grass hay at 2.5% BW in order to obtain baseline measurements. Beginning on day 2, horses were fed hay at approximately 60% of their maintenance DE requirements [6], which translated to 1% BW, each split evenly between two meals at 0700 and 1600 h. Horses had ad libitum access to water and were fed a ration balancer (ProAdvantage Grass Balancer, Progressive Nutrition) at 0.001% BW at 0700 h each day to ensure all vitamin and mineral requirements were met for adult horses at maintenance [6]. Horses were hand-walked twice daily for 30 minutes immediately prior to receiving their meal.

2.2 Time to Consumption, Dry Matter Intake, and Hay Analysis

Total time to consumption of the hay meal was measured on days 14 and 28 using a stopwatch. The stopwatch was started when the horses began eating their hay meal and was stopped when the horses finished their hay meal. Dry matter intake rate (DMIR) was determined by dividing the total amount of hay consumed (kg) by the total time to consumption (h).

Multiple small-square bales of grass hay were cored (2 x 51 cm) and combined to determine forage nutritive value. Samples were combined (n=5) and analyzed for forage nutritive value by a commercial forage testing laboratory (Equi-Analytical, Ithaca, NY) using the following methods. Dry matter was determined by placing samples in a 60°C forced air oven for 24 h [144]. Crude protein was calculated as the percentage of N multiplied by 6.25 [144]. Neutral detergent fiber and ADF were non-sequentially measured using filter bag techniques [129,130]. Starch, water soluble carbohydrates, and ethanol soluble carbohydrate were measured using techniques described by Hall et al. (1999). Mineral concentrations were determined (Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma Radial Spectrometer, Thermo Instrument Systems Inc., Waltham, MA) after microwave digestion (Microwave Accelerated Reaction System, CEM, Mathews, NC). Equine DE was calculated using an equation developed by Pagan (1998).

2.3 Morphometric and Ultrasound Measurements

On days 1, 14, and 28 of data collection, horse bodyweight, BCS [81], morphometric measurements, and ultrasound measurements of rump fat, longissimus dorsi (LD) muscle depth, and LD thickness were taken. Bodyweight was measured using a livestock scale (Fairbanks Scales, Kansas City, MO). Morphometric measurements were taken by trained personnel and included neck circumference located halfway between the poll and withers [145], girth circumference at the base of the mane hairs, and a cresty neck score on a scale of 0 to 5 [145]. Rump fat was measured 5 cm lateral from the midline on both the left and right sides of the rump, while the LD muscle depth and thickness was taken 5 cm lateral from the spinous processes between the 12^{th} and 13^{th} ribs [146]. Based on rump fat, the following equation was used to determine extractable fat [147]:

$Y = 8.64 + 4.70X$; where Y= percent extractable fat and X= cm of rump fat

2.4 Blood Sampling and Laboratory Analysis

Jugular catheters were placed approximately 2 h prior to blood sampling on days 1 (baseline), 14 and 28. Blood samples were drawn one hour prior to feeding (baseline), immediately after the morning meal (0700 h) was fed, and every 30 min for the next 3 h. Sampling continued hourly until the evening feeding at 1600 h. The procedure was then repeated after the evening meal and stopped at 0600 h the following day (n = 30). Approximately 10 mL of blood was drawn from each horse at every sampling using a 20-G syringe. Catheter lines were flushed with 10 mL of saline, followed by 10 mL of heparinized saline. Blood samples were then aliquotted into sterile Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ) and placed on ice for transport to the laboratory. Upon arrival in the laboratory, samples were immediately centrifuged at 4º C for 15 min at 2,000 *g* in order to separate serum and plasma. Plasma was collected and aliquotted into micro vials and frozen immediately at -20ºC until the laboratory analysis could be completed.

Glucose concentrations were determined using a spectrophotometric glucose assay (Coat-a-Count, Diagnostic Products, Los Angeles, CA) in duplicate. The interassay and intra-assay coefficients of variation were 5.5 and 3%, respectively. Serum insulin concentrations were determined using a commercially available radioimmunoassay (Coat-a-Count PITKIN-9, Diagnostic Products, Los Angeles, CA) that had been validated for equine plasma [148]. All samples were run in duplicate. The assay used ^{I25}I-labeled bioactive human serum as a tracer molecule. The interassay and intra-assay coefficients of variation were 1% and 11%, respectively. Plasma cortisol concentrations were determined using commercially available RIA kits (Coat-a-Count PITKCO-10, Diagnostic Products, Los Angeles, CA) in duplicate. The assay used ^{I25}I-labeled bioactive human serum as a tracer molecule. The interassay and intra-assay coefficients of variation were 1% and 9%, respectively. All tubes were counted with a Packard Cobra II Gamma Counter (Packard Biosciences, Boston, MA).

2.5 Statistical Analysis

Bodyweight, BCS, morphometric measurements, and ultrasound measurements were analyzed using the Proc Mixed procedure of SAS (SAS Institute, Cary, NC; version 9.3). The model included day, treatment, gender, and the day x treatment interaction. Area under the curve (AUC) for glucose (AUCglu), insulin (AUCins) and cortisol

(AUCcort) were analyzed using the trapezoidal method. Area under the curve was calculated for a period of 8 h after each feeding, the approximate time that horses return to baseline [149]. Total time to consumption, AUC, and average, time to peak, and peak values for insulin, glucose and cortisol were analyzed using the Proc Mixed procedure of SAS. The model included day, treatment, and day x treatment. To confirm effects of treatments and feeding (morning vs. evening), results from days 14 and 28 were isolated and analyzed separately. The data was analyzed using the Proc Mixed procedure of SAS, with the model including day, feeding, and day x feeding. All models included day as a repeated effect. Data were checked for normalcy using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Results were considered significant at $P \leq .05$.

3.0 Results and Discussion

3.1 Forage Nutritive Value, Time to Consumption, and Dry Matter Intake

Forage nutritive values for the grass hay and ration balancer are listed in Table 4. When compared to a national hay nutritive value database [133] the hay was within or near normal ranges for all nutrients tested for grass hay.

The horses feeding from the FLOOR took less time to consume their hay meal compared to horses feeding from the NET ($P < 0.001$). The mean total time to consumption for horses feeding from the FLOOR and NET were 123 (SE ±13 min) and 179 min (SE ±13 min), respectively. No differences were observed between morning and evening feedings ($P = 0.5895$). Dry matter intake rate was affected by treatment ($P = 0.0012$). Mean DMIR was 1.42 and 0.87 kg/ h (SE ±0.1 kg/h) for horses feeding from the FLOOR and NET, respectively.

The differences in DMIR between horses feeding from the FLOOR and NET are similar to results found by Glunk et al. (2013) who determined that DMIR from horses feeding from the stall floor was 1.49 kg/h compared to the same slow-feed hay net that resulted in 0.88 kg/h [150]. The DMIR were similar, even though horses in the previous study were fed hay at 2.0% BW and not a restricted diet. The current study confirms the effectiveness of slow-feed hay nets at extending total time to consumption and slowing DMIR. These characteristics are important when managing stalled horses, and especially when feeding a restricted diet. Increasing the time horses spend foraging each day promotes gut health and hindgut fermentation [11] and has been shown to reduce stereotypical behaviors [151] and the incidence of colic [139].

Previous research has shown that horses can acclimate and adapt to different amounts of feeding time. Longland et al. (2011) observed that ponies were able to increase their daily DMIR over a period of 6 weeks, while Glunk et al. (2012) determined that horses were able to increase their DMIR as the grazing period was reduced from 24 to 3 hours [119,122]. In the current study, there was an effect of day. Horses on day 28 took less time to consume their hay meal compared to day 14 (P= 0.047). These results indicate horses can acclimate to different feeding and management strategies over time.

3.2 Bodyweight, BCS, and Morphometric and Ultrasound Measurements

All horses lost bodyweight during the trial (P < 0.001); however, bodyweight loss was not affected by treatment (P = 0.326; Table 5). On average, horses on the FLOOR lost 32 kg (±7.4 kg), while horses on the HN lost 40 kg (±7.4 kg). There was no difference in horses' BCS, girth circumference, neck circumference, cresty neck score, average rump fat, extractable fat or LD depth between days 1 or 28 ($P \geq 0.42$), or between treatments ($P \geq 0.13$). There was an effect of day on LD thickness for horses feeding from the NET. Longissimus dorsi thickness on d 28 was less than LD thickness measured on d 1 (P =0.0257). No difference in LD thickness was observed from horses feeding from the FLOOR between days 1 and 28. The absence of significant differences in LD depth from day 1 to day 28 indicates that even though horses lost a significant amount of bodyweight, it is likely that most of the mass lost was as fat versus muscle, as muscle loss would have likely impacted LD depth measurements.

Caloric intakes were designed to be at approximately 60% of DE for adult, maintenance horses in order to achieve a reduction in one BCS unit in one month [6]. Heusner (1993) estimated an change one unit of BCS equaled 16 to 20 kg for mature horses [152]. Recently, Martinson et al. (2014) found that the differences between each unit of BCS averaged 15, 10 and 17 kg for Arabians, ponies and stock horses, respectively. In the current study, horses lost 32 to 40 kg of bodyweight in 28 days, but the BCS did not change between day 0 and 28. One potential reason for the inability to detect a change in BCS is the system itself. The BCS systems evaluates adipose tissue in six areas, including the ribs, behind the shoulder, along the neck and withers, in the crease of the back, and tailhead [81]. Most of the horses in the current study had adipose tissue in the lower abdominal area, a region not considered when assessing horse BCS.

Other measurements of adipose tissue deposits and muscle composition include girth and neck circumference, cresty neck score, rump fat, and LD thickness and depth. With the exception of LD thickness, none of these measurements changed over the 28 d period in the current study. This is similar to Dugdale et al. (2010) who found that neither rump fat depth nor neck circumference decreased with decreasing bodyweight when horses were fed hay at 1% BW [154]. However, Dugdale et al. (2010) did observe a decrease in girth circumference and LD depth with decreasing bodyweight, which was not observed in the current study. It is likely that horses in the current study lost adipose tissue in other regions, or the time fame was not long enough to see significant changes in these measurements. Gordon et al. (2009) found that a significant amount of rump fat was not lost until approximately week 6 of a diet and exercise trial [155].

3.3 Postprandial Metabolite Patterns

Results from days 14 and 28 were similar ($P \geq 0.05$) among the postprandial metabolites measured; therefore, only results from days 1 and 28 will be presented and discussed. Average, AUC, peak, and time to peak (TTP) for glucose (glu) insulin (ins), and cortisol (cor) are shown in Figure 3 and Table 6.

Average glucose was affected by day ($P < 0.0001$). Average glucose values increased from day 1 to 28, showing a possible effect of switching horses from free-choice hay to meal feeding. There was no effect of treatment or day of sampling on AUCglu, PEAKglu, or TTPglu ($P \geq 0.08$). These results agree with previous researchers who found no effect on TTPglu when horses lost bodyweight [116] or were fed a restricted diet [149]. However, Pagan et al. (1999) found an increase over time for TTPglu when horses were fed a restricted diet, which was not observed in the present study.

Average insulin values and PEAKins were affected by day ($P \leq 0.0218$) and feeding ($P < 0.001$) with values being greater on day 28 compared to day 1 during the morning feeding. These results disagree with Buff et al. (2002) and Frank et al. (2006) who observed decreased insulin values in ponies after bodyweight loss. Time to peak insulin was affected by treatment ($P = 0.037$), with horses feeding from the FLOOR having a longer TTP compared to horse feeding from the NET. To determine whether baseline values differed between horse groups, results from days 14 and 28 were analyzed separately. Treatments were still different ($P = 0.0111$), indicating that the increased time to consumption from horses feeding from the NET likely caused the difference observed in TTPins. AUCins and TTPins were not affected by day ($P \geq 0.330$), and average insulin values, AUCins, and PEAKins were not affected by treatment ($P \geq 0.6548$).

Figure 1 shows a difference in metabolic response between morning and evening feedings. To evaluate this further days 14 and 28 only were analyzed, and treatments were isolated. There was an effect of morning vs. evening feeding observed for horses on both the NET and FLOOR treatments when analyzed for AUCglu ($P = 0.0188$ and 0.0121, respectively) and at least a trend seen for AUCins ($P = 0.0672$ and 0.0056, respectively). Also significantly affected was ($P \leq 0.05$): average cortisol, average insulin, PEAKins, TTPglu, and PEAKglu.This effect is likely due to the addition of the ration balancer to the morning feed ration. On average, horses were fed 0.56 kg of the ration balancer each morning. In the current study, the feeding effect of the ration balancer appears to cause a similar increase in blood glucose and insulin observed after a concentrate meal is fed [116]. This effect is surprising, as ration balancers are not typically considered a source of calories. It is possible the increase in blood glucose and insulin from the addition of the ration balancer is exacerbated by the restricted diet.

Only PEAKcor was affected by treatment (P = 0.0207) with horses feeding from the FLOOR having greater peaks compared to horses feeding from the NET. Horses feeding from the FLOOR took less time to consume their hay meal compared to horses feeding from the NET. When finished with the hay meal, horses feeding from the FLOOR were still able to see horses feeding from the NET. This likely resulted in a stressful situation which may have led to the increase in PEAKcor levels for these horses. Average cortisol levels and AUCcor were affected by day ($P \leq 0.0102$). Average cortisol values and AUCcor were greater on day 1 compared to day 28. Several other researchers have observed a decrease in cortisol over time when horses were subject to a restricted diet [149,155,157]. Sticker et al. (1995) determined that cortisol levels decreased by day 9 when horses were fed a restricted diet. This finding is slightly counterintuitive, as it would be expected that a decrease over time in average cortisol may help decrease average glucose over time as cortisol is an important glucose regulator [86,106,112,116]. However it is likely that there are many other factors affecting the elevated glucose levels, such as meal-feeding and restricted diet, leading to the results seen in this study.

Some researchers have reported a daily circadian rhythm in cortisol with peaks observed in the morning and valleys observed in the evening [107,116,158]. This pattern was not observed in the present study and may be due to meal feeding, the restricted diet or housing the animal indoors altering their light perception. Altered light perception has been found to impacts cortisol circadian rhythms in humans and rats [107,159,160].

4.0 Conclusion

All horses lost bodyweight when subjected to the restricted diet, however; no differences were observed between horses feeding from the FLOOR and NET. The horses feeding from the FLOOR took less time to consume their hay meal compared to horses feeding from the NET. There were no differences in BCS, morphometric measurements, or ultrasound measurements except LD thickness in horses feeding from the NET. Only time to peak insulin and peak cortisol levels were affected by treatment with horses feeding from the NET having lower values compared to horses feeding from the FLOOR. Averages of the metabolites measured, AUC cortisol, and peak insulin were affected by day. Glucose and insulin values increased while cortisol levels decreased during the study. The effect of morning vs. evening feeding was observed for insulin and was attributed to the addition of a ration balancer to the morning feed ration.

The results of this study indicate that restrictive feeding is an effective method of inducing weight loss. Additionally, slow-feed hay nets show promise in decreasing the stress of horses that are placed on weight loss regimens, perhaps leading to a better overall mental and physiological state.

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Appendix

Table 1. Initial herbage intake and percent reduction of horses grazing four cool-season grass species with and without the use of a grazing muzzle in 2012.

Species	Muzzle	Initial Herbage Mass consumed	Reduction
		%	
Kentucky bluegrass	Without	69	
	With	31	38*
Meadow fescue	Without	79	
	With	49	30*
Perennial ryegrass	Without	66	
	With	22	45*
Reed canarygrass	Without	47	
	With	43	4
Average Percent Reduction			29

*Indicates significant effect of muzzle at $P < 0.05$

Table 2. Initial herbage intake and percent reduction of horses grazing two cool-season grass species with and without the use of a grazing muzzle in 2013.

Species	Muzzle	Initial Herbage Mass consumed	Reduction
		%	
Kentucky bluegrass	Without	89[a]	30*
	With	59[c]	
Reed canarygrass	Without	80[b]	31*
	With	49[d]	
Average Percent Reduction			30

[a-d] Numbers within columns with different superscripts indicate significant difference among treatments

*Indicates significant effect of muzzle at $P < 0.05$

Table 3. Forage nutritive value (means ± SE) of the mixed, mostly grass hay fed to adult horses during trial 2 (Chapter III).

Nutrient[a]	**Content**
	%
DM	91.8 (±0.09)
CP	14.3 (± 0.21)
ADF	36.5 (± 0.23)
NDF	56.8 (±0.24)
Starch	1.5 (±0.15)
WSC	8.6 (±1.07)
ESC	6.5 (±0.12)
Ca	0.68 (±0.03)
P	0.35 (±0.003)
	Mcal/lb
Equine DE	1.0 (±0.01)

[a]DM, dry matter; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSC, water soluble carbohydrates; ESC, ethanol soluble carbohydrates; Ca, calcium; and P, phosphorus ,DE, equine digestible energy.

Table 4. Nutritive value of grass hay and ration balancer fed to adult horses.

Nutrient[a]	Hay Content	Ration Balancer Content
	% DM	
DM	91.8	89.4
CP	12.5	39.1
ADF	38.2	9.5
NDF	62.9	18.3
Starch	1.4	4.8
WSC	11.0	10
ESC	5.8	7.6
Ca	0.47	2.1
P	0.31	1.09
	Mcal/kg	
Equine DE	2.0	1.5

[a]DM, dry matter; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSC, water soluble carbohydrates; ESC, ethanol soluble carbohydrates; Ca, calcium; and P, phosphorus; DE, equine digestible energy.

Table 5. Bodyweight, BCS, morphometric measurements, and ultrasound values of horses fed a restricted diet from either the stall floor (FLOOR) or a slow-feed hay net (NET) on days 1 and 28.

Treatment	**Day**	**Body Weight (kg)**	**BCS**	**Neck Circumference (cm)**	**Girth Circ. (cm)**	**Cresty Neck Score**	**Average Rump Fat (cm)**	**% Extractable Fat**	**LD Thickness (cm)**	**LD Depth (cm)**
FLOOR	1	565a	6.7	39	76	1.5	1.6	13	0.62^{a}	4.0
	28	565b	7.0	40	76	1.5	1.8	14	0.51^{a}	3.8
NET	1	537a	7.2	41	76	2.3	2.3	16	0.56^{a}	4.0
	28	525b	7.0	41	75	2.0	1.7	13	0.47^{b}	4.0

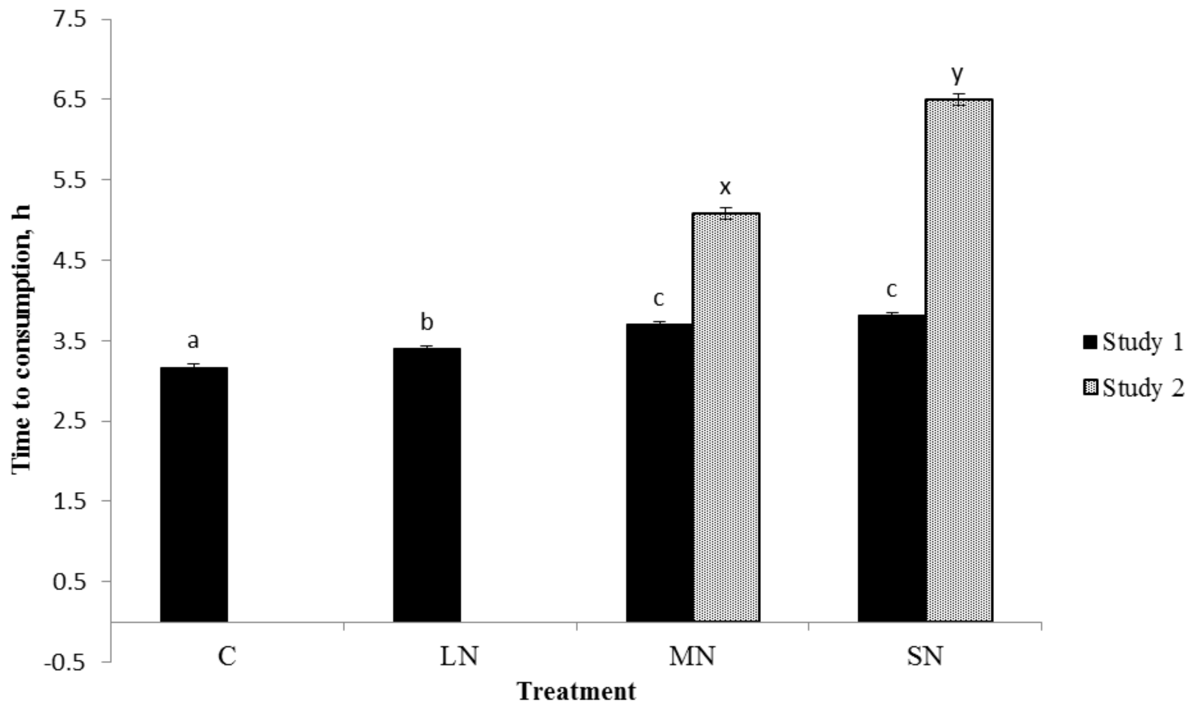
^{a,b} Means without a common superscript within a column differ ($P \leq 0.05$).

Table 6**.** Average (Avg) values, area under the curve (AUC), peak, and time to peak (TTP) for blood glucose, insulin and cortisol for horses fed a restricted diet at 0700 h (morning) and 1600 h (evening) from either the stall floor (FLOOR) or a slow-feed hay net (NET) on days 1 and 28

		FLOOR				NET					
		Day 1		Day 28		Day 1		Day 28			
		Feeding								*P*-value	
		Morning	Evening	Morning	Evening	Morning	Evening	Morning	Evening	Treatment	Day
Glucose	Avg (mg/dL)	106^{y}	108^{y}	120^{x}	113^{x}	105^{y}	100^{y}	115^{x}	116^{x}	0.4695	<0.0001
	AUC	4,506	6,644	5,064	7,246	4,397	5,948	4,857	6,939	0.5552	0.2430
	Peak (mg/dL)	120	136	143	124	117	111	128	129	0.16	0.28
	TTP (mins)	112	97	187	135	172	112	217	82	0.67	0.08
Insulin	Avg (IU)	12.1^{y}	13.0^{y}	20.6^{x}	10.0^{x}	12.3^{y}	12.7^{y}	18.1^{x}	9.2^{x}	.8229	0.0218
	AUC	514	785	911	579	500	709	733	506	0.6548	0.3832
	Peak (IU)	15^{y}	16^{y}	44^{x}	16^{x}	18^{y}	18^{y}	34^{x}	15^{x}	0.82	0.0277
	TTP (mins)	120^{a}	90^{a}	187^{a}	120^{a}	112^{b}	97^{b}	106^{b}	67^{b}	0.037	0.330
Cortisol	Avg (µg/dL)	6.1^{x}	6.7^{x}	5.1^{y}	3.7^{,y}	3.0^{x}	2.6^{x}	2.9^{y}	2.5^{y}	0.0642	0.0002
	AUC	260^{x}	387^{,x}	198^{,y}	217^{,y}	123^{x}	156^{x}	115^{y}	140^{,y}	0.0684	0.0102
	Peak (µg/dL)	5.8^{a}	3.3^{a}	8.2^{a}	5.0^{a}	4.3^{b}	3.1^{b}	4.4^{b}	3.9^{b}	0.0207	0.0679
	TTP (mins)	82	97	67	142	120	97	112	67	0.9568	0.9389

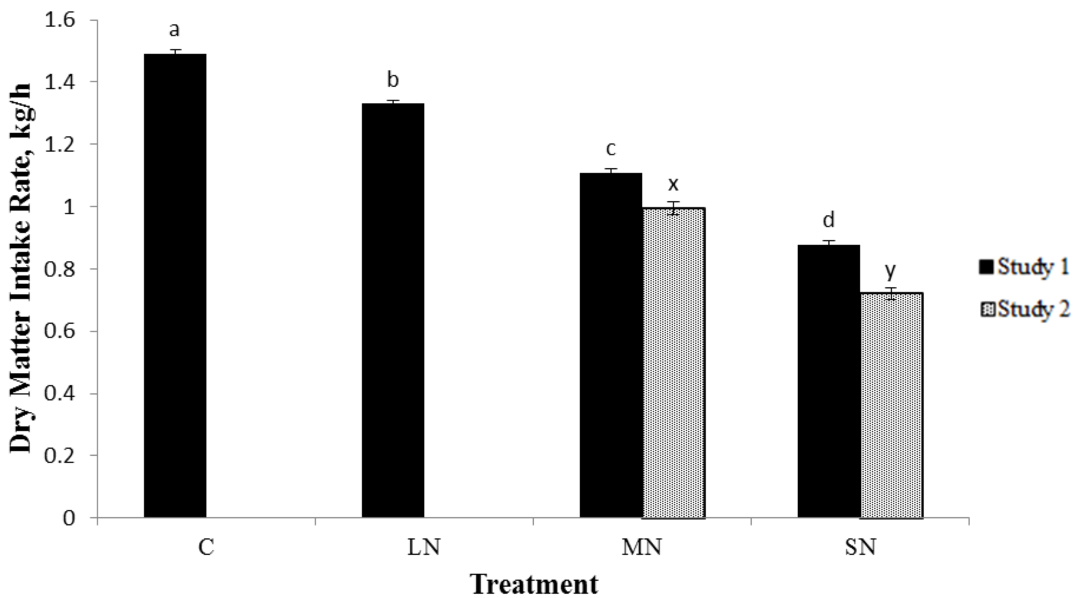
[a,b,c] Within a row, means without a common superscript differ by treatment ($P \geq 0.05$)
[x,y,z] Within a row, means without a common superscript differ by period ($P \geq 0.05$)

Figure 1. Time to consumption (h) of horses feeding from the control (C), large net (LN), medium net (MN) and small net (SN) during study 1 (solid bars) and study 2 (textured bars).



[a,b,c]Bars without a common letter differ ($P \leq 0.05$)
[x,y]Bars without a common letter differ ($P \leq 0.05$)

Figure 2. Dry matter intake rate (kg/h) of horses feeding from the control (C), large net (LN), medium net (MN) and small net (SN) during study 1 (solid bars) and study 2 (textured bars).



[a,b,c]Bars without a common letter differ ($P \leq 0.05$)
[x,y]Bars without a common letter differ ($P \leq 0.05$)

Figure 3. Changes in glucose, insulin, and cortisol over a 24 h sampling period when horses were fed a restricted diet at 0700 and 1600 h from either the stall floor (FLOOR) or a slow-feed hay net (NET) on days 1 and 28.