ABSTRACT

DOWLER, LAUREN ELIZABETH. Prediction of Hourly Pasture Dry Matter Intake in Horses. (Under the direction of Paul David Siciliano.)

Establishing hourly dry matter intake (DMI) rates for horses grazing pasture would allow for calculation of the required length of time for horses to consume only their daily caloric requirement; thereby preventing wasteful overconsumption of pasture. Unfortunately, estimates of hourly pasture DMI rates are scant. The objective of this two-part study was to use an herbage mass (HM) reduction method to determine pasture DMI over an 8-hr period (experiment 1- EXPT1) and to test the validity of these estimates (experiment 2 – EXPT2). Pasture DMI in EXPT1 was measured using eight horses of light horse breeding (7 mares, 1 gelding; mean ± body weight 576 ± 32 kg; mean age 15.6 ± 6.8yr) at three separate times throughout the year (October 2008, and February, and May of 2009 - hereafter referred to as periods 1, 2, 3, respectively). Horses grazed for two consecutive 4-h periods in each of two separate 5m x 5m cells following 12h of feed restriction. The HM of each cell was determined prior to and after each 4-hr grazing period and was used to determine hourly DMI rate. Mean pasture DMI rate over the entire 8-h grazing period was 0.166, 0.088, and 0.108 ± 0.013 kg DM · 100 kg BW^{-1} · h^{-1} in periods 1, 2, and 3, respectively. Mean DMI rate over 8h was different across periods (period 1 > 2 and 3; $P < 0.005$) and cells (cell A > B; $P < 0.001$). Experiment 2 immediately followed EXPT1 in each period. The same horses from EXPT1 were randomly assigned to one of two treatments: unrestricted grazing (UNRES; n=4) or restricted grazing (RES; n=4) for the 42d. Horses in the UNRES group grazed continuously; horses in the RES group grazed only for a time period calculated to allow for consumption of daily DE_m requirement only. Daily DE_m was calculated using the hourly

DMI rates determined in EXPT1, pasture DE concentration, and daily maintenance DE requirements. Body weight was monitored weekly. Mean BW increased over time (P = 0.013) by 7.9 ± 17.91 kg in period 1 and decreased over time in both period 2 ($P < 0.001$) and 3 ($P < 0.001$) (11.5 ± 16.98 and 44.44 ± 17.45 kg, respectively), but neither treatment nor treatment x time interaction were significant in any period. When extrapolated to a 15 h grazing time each day, the hourly DMI estimates correspond to a daily DMI of 2.49, 1.32, and 1.62% BW for periods 1, 2, and 3, respectively. While the estimate from periods 1 and 3 seem reasonable and are within the range of the NRC (2007), the estimate from period 2 seems low, and is not within the range (NRC 2007). However, the change in BW in each period of EXPT2 suggests that hourly intake rate likely varies from day to day as well as with changes in environmental variables, season, and forage characteristics. Withholding feed prior to EXPT1, could have led to atypical hourly DMI rates. In future studies estimating hourly pasture DMI, it may be useful to alter horse management techniques. While this data suggests that herbage mass reduction is a useful tool for determining DMI, it may be most accurate under specific conditions. Studies comparing the estimates of hourly DMI rate obtained through different methods such as markers, short term weight changes, and measurement of fecal outputs and known DM digestibilities would be helpful in determining the most useful and accurate method to obtain estimates in the future.

Prediction of Hourly Pasture Dry Matter Intake in Horses

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Animal Science

Raleigh, North Carolina

2009

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DEDICATION

This thesis is dedicated to my mom and dad, Lois and David, for providing me with the best childhood I could have imagined and for instilling in me a not only a good work ethic, but also a positive attitude and a curiosity for science and the world. It is also dedicated to my sister, Jorden, for her support and endless humor and to my Grandmother, Lena, for encouraging me to do things I didn't know I could handle. Lastly, to my Grandfather, Don, for inspiring my interest in agriculture and for showing me the value of hard work. I love you all more than the, "whole wide world."

BIOGRAPHY

Lauren Elizabeth Dowler grew up on a small farm in the rural town of Meadville, Pennsylvania. She was introduced to agriculture and farming as a child and can remember loving animals from a very young age. Some of her fondest childhood memories are those of helping her mom and dad manage a small herd of Holstein dairy cows and eventually a group of Welsh Thoroughbred ponies. She looks back especially fondly on spring and summertime on the farm when foals were being born and every moment could be spent with the animals. She graduated from Meadville Area Senior High in 2003 and immediately began her study of Animal Science and Equine Science at The Pennsylvania State University. Under the advisement of Dr. Gabriella Varga, Lauren had her first experiences with nutrition research and became interested in pursuing a graduate degree. She graduated from Penn State in 2007 and subsequently moved to Raleigh, North Carolina to pursue a Master's in Animal Science and Nutrition under the direction of Dr. Paul Siciliano.

ACKNOWLEDGMENTS

I would like to thank a number of individuals who, in various ways, have assisted with the completion of this project:

Dr. Paul Siciliano, for his advising, both professional and personal, for his dedication to this project, and for always having an interesting "bunny trail" to explore;

Dr. Shannon Pratt-Phillips, for her advising and for bringing graduate school to life;

Hannah Bowers and Daniel Fordham and the other undergraduate students who not only assisted with sample collection and management of the experiment, but more importantly, brought positive attitudes, humor, encouragement, and hours of companionship regardless of the situation and with whom close and lasting friendships were formed;

Kelly Owens, for her friendship and support through our graduate program;

Alaina Parsons, for being so generous with her time, and for always brightening early mornings with a, "mornin' sunshine!";

Missy Murphy for her endless patience in helping me "learn the ropes" of labwork;

Marian Correll for all of her help and for being the "grad school mom";

The various graduate students in the Department of Animal Science, without whose support and guidance I would have long ago lost my sense of humor;

And, my dog, Nessie, for her unwavering happiness and companionship, and for laying at my feet while I completed this thesis;

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CHAPTER 1

REVIEW OF CURRENT LITERATURE

Grazing is a natural behavior of feral, semi-feral, and domesticated horses. As such, a number of investigators have observed a variety of different types of horses in hopes of elucidating both the general grazing behaviors as well as the factors that affect grazing behavior and control of appetite. It has been observed that grazing behavior varies depending upon a number of factors (i.e. hours of daylight, season of the year, etc). These factors are numerous and while the aim of many investigators is to determine the specific effects of these factors, it is important to realize that equine behavior is both highly complex and variable. Thus equine behavior involves the intricate interaction of each of these variables at any given time. What follows is a review of the factors that have been observed to contribute to the grazing behavior of horses.

Daily Grazing Time and Frequency of Grazing

Estimates of daily time spent grazing in both semi-feral and domesticated horses range from 10 to 17.2 hours per day with 15 to 20 grazing periods, which corresponds to about 40 to 72% of a 24-hr period (Fleurance et al., 2001; Frape, 2004; Lewis, 1995). Similarly, Lewis reports that for mature horses, tame and feral, on pasture with ample forage and no other feed during mild weather, grazing time ranges from 40 to 60% of a 24-hr period (Lewis, 1995). Furthermore, Lewis reports that grazing comprises 60 to 80% of daylight hours, with the most continuous grazing periods taking place in early morning, late afternoon and evening, and the middle of the night (Lewis, 1995).

It has also been observed that horses graze in a diurnal pattern with an early morning and dusk component NRC (2007) and that seasonal changes in ambient temperature and day length alter the absolute ultradian and daily patterns (Berger et al., 1999). Sunrise and sunset were determined to impact the daily patterns over a period of one year in a group of semi-feral Przewalski horses (Berger et al., 1999). Yearling horses grazing in September and December, also showed a circadian pattern of grazing with a depression in grazing behavior just prior to sunrise (4 to 6 a.m.) and just after sunset (6 to 8 p.m.) (Hansen et al., 1987b). Houpt, (1990) observed free ranging horses spending about 75% of the day and half of the night grazing. Assuming "day" and "night" were divided equally into two 12-hour periods, this would equate to 15 hours of grazing time in 24 hours, which is within the range of other estimates.

Separately, Fleurance et al. (2001) found that heavy-breed mares grazing heterogeneous grass pastures from April (spring) to October (autumn) spent nearly 75% of the night grazing and only about 50% of the daytime grazing (Fleurance et al., 2001). However, Fleurance likely separated daytime and nighttime into unequal periods, since they also report that nocturnal feeding comprised 38% of the total grazing time. Berger et al., (1999) also found that activity and feeding during daytime was higher than at night, with the exception of the hottest time of year when both activity and feeding were higher at night than during the day (Berger et al., 1999). Horses presumably spent more time standing idle so that high temperatures and flying insects could be avoided (Berger et al., 1999). Thus, heat and annoyance from flying insects may also affect time spent grazing (NRC, 2007). This is

supported by the finding that Camargue and Przewalski's horses rested during peak heat periods of the day and that any grazing during the heat of the day was considered atypical and was concluded to be an indicator of undernourishment (Klimov, 1988; Mayes and Duncan, 1986). Peak feeding by pastured Welsh ponies was observed to occur between 5:00 to 9:00 pm (Crowell-Davis et al., 1985). Lastly, Frape reported on a study conducted in North Carolina, where mares spent up to 90 % of the daylight and 76 % of the dark grazing (Frape, 2004). Overall, these estimates suggest that more time is spent grazing during the daylight than is spent during darkness.

Season

Grazing time has also been found to vary with season. As pasture availability increases, typically in the spring and fall, a decrease in time spent grazing has been observed (Arnold and Grassia, 1982). With grass senescence, typically in summer and winter, a decrease in both pasture intakes (1.8 – 2.0 kg DM/ 100 kg BW) and grazing time (52 – 58 % of the day) have been observed (Shingu et al., 2000). Berger et al., (1999) determined feeding accounted for 62 % of total activity in the spring, whereas it only accounted for 40 % of total activity in the summer, with a year round average of 52 % of activity (Berger et al., 1999). Hansen et al. (1987b) also saw seasonal differences in grazing time of yearlings grazing Bermudagrass pasture in September and rye-ryegrass pasture in December. In September, yearlings spent 16.3 hours (67.8 %) of a 24-h period grazing, whereas in December they spent 13.8 hours (57.2 %) of a 24-h period grazing (Hansen et al., 1987b). However, since different forages

were used to determine grazing time in September and December, the decrease of grazing time in December may have been due to differences in forage characteristics.

Weather

Along with season, weather and environmental conditions have also been found to affect grazing behavior. Cymbaluk and Christison (1990) report that the five climactic variables of a horse's microclimate are: ambient temperature, relative humidity, precipitation, wind velocity, and solar radiation (Cymbaluk and Christison, 1990). Of these, the most important stressor is ambient temperature (Cymbaluk and Christison, 1990). Effective ambient temperature is a way to express all variables as a composite (Cymbaluk and Christison, 1990). High temperatures, winds, and heavy rainfall result in decreased time spent grazing (Crowell-Davis et al., 1985; Houpt, 1990) whereas cold conditions (Houpt, 1990) or increased relative humidity have been found to increase grazing time (Rogalski, 1974). Horses have also been observed to spend more time feeding during cold weather and less during hot weather (Booth, 1998).

Thermostatic control of appetite likely has an impact on DMI during different weather conditions. When the weather is cold or wet, animals' maintenance needs increase in order to maintain body temperature. Hence, their intake increases (Houpt, 1990). However, even with an increase in intake, animals may not be able to overcome the effects of cold wet weather. In one study, mares were fed at 150% of the recommended energy intake during cold wet weather, but still lost weight (Kubiak et al., 1988). It has also been suggested that at

low environmental temperatures, fiber digestion may improve in order to increase heat increment and aid in maintenance of body temperature (Stahly and Cromwell, 1986). Conversely, in hot weather, horses decrease feed intake and increase water consumption in order to keep cool (Houpt, 1990). While feed intake by horses may decrease by 15 to 20 % during hot weather, compensatory responses may act to increase diet digestibility (Cymbaluk and Christison, 1990). Therefore, the type of feed provided to horses in hot weather is also important. The heat increment associated with diets can aggravate or minimize heat stress. Fibrous feeds, such as hay, often aggravate heat stress as the heat increment of these feeds is relatively high in comparison to the heat increment of grains or fat (Cymbaluk and Christison, 1990).

Forage Characteristics

Forage characteristics have been observed, in many cases, to greatly impact selection of forage, overall DMI, and DMI rate. Horses are selective grazers and, like most herbivores, they do not simply eat from plants that are in the greatest abundance, but instead base their consumption on palatability as well as availability (Houpt, 1990). Until preferred forages are depleted, horses will eat only a few of many species available (Lewis, 1995). These preferences result in spotty grazing, with some areas grazed completely to the ground (i.e. lawns) and other areas of tall ungrazed forage (i.e. “roughs”), leading to incomplete utilization of pasture forage (Lewis, 1995). Proper pasture and grazing management can help to eliminate these problems.

It has been suggested that DMI rate varies with forage density, quality, digestibility, palatability and other characteristics, as well as with appetite and size of the horse (Frape, 2004; Lewis, 1995; NRC, 2007). Although horses on pasture for 24 hours will normally spend most of their time grazing, they can potentially consume a sufficient quantity to meet their maintenance energy needs in 4 to 5 hours daily, if adequate quantities of highly nutritious forage are available (Lewis, 1995). However, Lewis suggests that the rate of consumption would be less the more hours the horses are on the pasture each day (Lewis, 1995). Researchers studying horses pastured on warm-season species and perennial ryegrass, concluded that horses needed to graze for approximately 17 hours daily to meet their nutritional needs (Gallagher and McMeniman, 1988; Hughes and Gallagher, 1993). These hypotheses regarding required grazing time to consume daily requirements align well with the objective of the current study to determine hourly DMI rate.

Forage characteristics can alter the palatability of the forage and hence, both the rate at which it is consumed as well as the daily DMI. Forage maturity is likely one of the most important factors affecting selection by horses as well as palatability. Changes in forage maturity coincide with changes in moisture, fiber, and digestibility, among other fractions. As forages mature, their palatability and digestible nutrient content decrease rapidly. Hence, the more immature the plant, the more nutritious and palatable (Lewis, 1995). As a result of the mature plants' reduced palatability, horses decrease intake of these mature forages, and instead will overgraze the more palatable, immature plants (Lewis, 1995). Fleurance et al., (2001) suggests that horses select herbage on the basis of stage of growth rather than

botanical species. This is in agreement with other reports of grazing animals preferring green (young) herbage instead of brown (old) herbage (Arnold and Grassia, 1982). While horses may eat young, green herbage more often than more mature, brown forage, it is likely that this preference has more to do with the maturity of the herbage than it does with color. This is supported by the findings of Fleurance et al., (2001) who found that heavy-breed mares preferred to graze shorter grass lawns (immature forage) versus taller grasses (mostly at the reproductive stage) and hence, selected a diet with a relatively higher digestibility (Fleurance et al., 2001).

Fiber content is a forage characteristic that can greatly affect the digestibility of forages and typically varies with changes in maturity. Changes in maturity are often associated with season, which is supported by the finding that nutrient content is highest during spring and fall growth (Lewis, 1995). Furthermore, the acid detergent fiber (ADF) percentage of forage being grazed by semi-feral horses was found to vary from 30% in the winter to 55% in the spring (Houpt, 1990). Fleurance et al. (2001), found that horses preferred to graze short grass lawns rather than tall grasses. In this experiment, the short grass lawns tended to have less cellulose (26.8 ± 2.2 vs. 30.8 ± 1.6) and neutral detergent fiber (NDF) (62.1 ± 2.8 vs. 67.1 ± 2.9 %) than the tall grasses. Additionally, Frape (2004) suggests that if ingesta derived from very coarse, poorly digested, long-fibrous feeds, (all characteristics of mature forage) is present in large amounts, it will be retained longer in the large intestine and depress the daily DMI. This agrees with the findings of Moffitt et al. (1987) that increased

herbage DMI are coincident with increased herbage quality, in terms of digestibility, and reduced cell wall content (Benyovsky et al., 1998).

While high moisture content typically suggests that a forage is less mature, a high moisture content can also potentially depress intake (Ince et al., 2005). The rye-ryegrass pasture used in the Hansen et al., (1987b) study to determine grazing time in December had a greater moisture content than the Bermudagrass pasture used in September. This increase in moisture may have suppressed grazing behavior by increasing gut fill and the feeling of satiety to the horses without as great an intake (Hansen et al., 1987b; Ince et al., 2005). Ince et al. (2005) suggests the relatively high moisture content (86.4 %) of the forage fed to a group of welsh ponies could have been a factor depressing intake.

Forage maturity can also affect digestible energy (DE) and protein content of grasses. Lewis noted decreases in DE and crude protein (CP) of one-third to one-half from mid-vegetative stage (2 to 4 weeks of growth and 2/3 mature height) to seed-forming maturity (12 weeks of growth) (Lewis, 1995). Decreases in DE and CP likely coincide with increases in fiber content of the forage. This is supported by Fleurance's findings, where the short grass lawns preferred by horses had a higher CP content and a lower cellulose and NDF content than the taller grasses that were not grazed as often (Fleurance et al., 2001). Furthermore, a number of investigators have reported that increased DMI are related to increased herbage quality in terms of digestibility (Moffitt et al., 1987), high sugar content (Rogalski, 1984) or increased CP content (Benyovsky et al., 1998).

This is supported by the finding that CP content can influence DMI. Fleurance et al. (2001) found a significant difference in the CP of the short grass lawns (12.8 ± 1.3 %) that horses preferred, versus the tall grasses (9.3 ± 1.2 %) where horses spent the least time grazing. Houpt (1990) reported that the variation in feral horses' diets through the seasons was potentially related to the CP content of the diet. The CP content of the diet was found to be 4 % in winter whereas it was 16 % in the spring. Hansen et al. (1987b) found that yearling horses' spot grazing of Bermudagrass pastures from March through September could also have been related to CP content of the diet. The CP content of the longer Bermudagrass (18.2 %) was lower than that of the shorter Bermudagrass "lawns" (20.6 %). However, the difference in CP between the two heights of Bermudagrass was most likely due to leaf : stem ratio and the immaturity of the forage (Hansen et al., 1987a).

Sward Height

Sward height is also a forage characteristic that may play a role in grazing preferences, although there have been conflicting results. Mesochina et al. (2000) offered three heights of pasture (6.6, 8.1, and 9.4 cm) to growing horses and found that grass height had no effect on DMI. Hughes and Gallagher (1993) also found that herbage dry matter (DM) intakes by horses did not change with increasing sward height.

Conversely, Hansen et al. (1987a) found that yearlings tended to select the immature forage of Bermudagrass "lawns," (areas of forage that are shorter and have been grazed heavily), that only represented approximately 38 % of total area, as opposed to the tall grass of

infrequently grazed “roughs.” Fleurance et al. (2001) also found that heavy breed mares preferred to graze short grass lawns (sward height ≤ 4cm) over taller grasses, even though the short grass lawns only represented 10 % of the grazing area. The mares spent about 70 % of their time grazing these short lawns regardless of the fact that they represented such a small percentage of the area (Fleurance et al., 2001). One hypothesis to explain the preference of short grass lawns versus patches of taller grass is that feces are more often deposited in the taller grass. Thus, animals may minimize grazing the taller grass to avoid intake of feces and/or gastrointestinal parasites (Fleurance et al., 2001). A different hypothesis is that if horses graze to maximize daily energy intake, the grazing of shorter, less mature forage could provide a greater amount of digestible nutrients (Fleurance et al., 2001).

Naujeck et al. (2005) had contrary findings when they conducted a study to investigate the effect of sward height on grazing preferences by horses grazing perennial ryegrass swards. They found that horses grazed longer and took more bites on patches with long grass (15 cm) than on those with short grass (< 4.5 cm) and that horses fed mainly on grass taller than 7 cm (Naujeck et al., 2005). Horses took more bites of forage at a length of 15.5 cm than on patches of 3.6 and 5.3 cm height and more bites on 8.2 cm patches than 3.6 cm patches (Naujeck et al., 2005). The horses in this trial visited short patches of 3.6 and 5.3 cm sward height only briefly whereas patches of 15.5 cm sward height were highly selected, followed by patches of 8.2 cm sward height (Naujeck et al., 2005).

Naujeck et al. (2005) postulates that one of the important influences on grazing behavior in horses is the requirement to optimize energy intake per bite within time and hence, horses select patches of forage with greater DM mass (kg DM/m^2). Furthermore, horses may avoid consuming soil, and thus prefer to graze taller grasses that are less contaminated (Naujeck et al., 2005). Although the horses in the study of Naujeck et al. (2005) visited patches of all heights, they showed a grazing preference for the taller heights. One potential reason for this visitation behavior was the difference in visual characteristics between the experimental field (square patches of forage at different heights) and fields where the forage appeared more "natural," like a field that has either not been grazed at all (homogenous forage height) or a field that had been grazed normally (lawns and roughs). The horses therefore may have focused more on the uncut, taller patches of grass with the more "normal" appearance. In addition, mowing forage (to obtain a certain height) likely changes the way it is perceived by the horse. Changes in texture, appearance, and aroma could alter the normal grazing behavior. Lastly, the range of sward height in this experiment only ranged from 3.6 to 15 cm. Therefore, it is possible that these sward lengths were not of adequate height to accurately represent the heights of forage a horse would prefer in a natural setting.

Other Factors

Differences in grazing time also exist in relation to gender, age, and breed differences of horses. Nash and Thompson (2001) observed fillies grazing for 66% of their time while colts grazed for 57 % of their time. Age may also have an effect on time spent foraging according to the results of Mesochina et al. (2000), who found that 2-year-old horses spent less time

foraging, but had a greater intake rate than yearlings. Shingu et al. (2000) observed differences in time spent grazing between native and non-native breeds. Hokkaido native horses were observed to graze for 13.8 hr ± 57.9 min whereas non-native light breed horses grazed for a slightly shorter time of 12.5 hr ± 31.3 min (Shingu et al., 2000).

Horses also appear to be stimulated to eat, in part, by visual contact of companion horses NRC (2007), a phenomenon of social facilitation (Houpt, 1990). Houpt and Houpt (1988) confirmed that horses isolated from direct visual contact of others were three times more active and spent 51.5 % less time eating compared to being housed together or separately but in visual contact of other horses (Houpt and Houpt, 1988). This may be due to the "antipredator strategy" of horses, where one horse acts as the lookout while the others graze (Houpt, 1990). Arnold and Grassia (1982) observed that in a herd of Thoroughbred mares with a 4 year old stallion at pasture, each horse was typically close to at least one other horse while grazing and that pairs of horses often graze and rest together. Furthermore, visibility of other horses, particularly in the afternoon, reportedly increased the time spent feeding by pony mares (Sweeting et al., 1985).

Overall, as is evident from the studies discussed in this review, a number of factors, and more importantly, their interactions greatly influence the day-to-day behavior of both feral and domesticated horses. Factors such as daylight, season, weather, gender, herd dynamics and companionship, and forage characteristics all interact to impact behavior, daily DMI, and likely, hourly DMI rate.

CONTROL OF APPETITE

There is conflicting evidence about the factors that control appetite and hunger in equines. Frape (2004) postulates that appetite and feed capacity of horses are regulated by five dominant and related factors: 1) the volume of different parts of the intestinal tract, 2) the rate of passage of the digesta, 3) the concentration of certain digestion products in the intestine, 4) the energy demands of the horse, and 5) the energy density and its chemical form in feed. It has also been suggested that the concentration of certain digestion products in the intestine seems to impact control of meal size and that the rate of passage will be modulated by the physical form of the feed (Frape, 2004). The normal feeding behavior of horses to consume several small meals throughout the day reflects both the low capacity of the stomach and even more directly, the switching-off mechanism of digestion products in the small intestine (Frape, 2004). Similarly, in horses, as in other animals, food intake is decreased by an elevation in osmolality in the stomach or the duodenum in proportion to the increase in osmotic pressure (Houpt, 1990). These findings support the theory that control of appetite is regulated by the interactions of many mechanisms, including digestive products, energy balance, gastro intestinal (GI) tract physiology, and hormonal control.

In a review of current literature, Havel (2001) discusses short-term and long-term signals that regulate food intake and energy homeostasis. Havel (2001) suggests numerous peripheral signals, both short-term and long-term, contribute to the regulation of food intake and energy homeostasis in humans. This may be one of the reasons why adult humans' (and animals) BW tends to remain within a relatively narrow range, despite large daily fluctuations. It has

been observed that nutrients such as amino acids and fatty acids as well as GI peptide hormones (i.e. cholecystokinin) are involved in short-term regulation of food intake. These short-term signals are primarily from the GI tract and are involved in sensations of satiety that lead to meal termination. However, these short-term signals are not sufficient to maintain energy homeostasis and body adiposity. Therefore, they must interact with long-term regulators (i.e. insulin, leptin, and potentially ghrelin) to maintain energy balance. In humans, insulin and leptin concentrations decrease during fasting, independent of body fat changes, ensuring that hunger is triggered before body energy stores are depleted (Havel, 2001). Therefore, short-term and long-term food intake and energy balance are regulated through distinct, but interacting, mechanisms (Havel, 2001). In humans, short-term signals such as nutrients and GI hormones primarily influence satiety and the size of individual meals, whereas the activity of long-term regulators of energy balance (i.e. insulin and leptin) is proportional to body adipose stores as well as energy consumption over longer time periods. Therefore, the long-term regulators help to maintain BW and adiposity by controlling food intake and energy expenditure. Together, the short- and long-term signals interact to regulate energy balance.

Hormonal Control

Adipose tissue and associated hormones also play a key role in control of appetite. Adipokines, proteins released from adipose tissue such as leptin and adiponectin, have been found to play a role in the regulation of energy metabolism (Radin et al., 2009). Various hormones, peptides, and other compounds have effects on control of appetite, but a review of

each of these is beyond the scope of this review. This review will focus on the effect of leptin, adiponectin, ghrelin, cholecystokinin, and peptide YY on the control of appetite.

Leptin likely plays a role in the regulation of energy expenditure since its administration induces greater weight loss than can be explained solely by the reduction of food intake (Havel, 2001). Circulating leptin concentrations are proportional to fat mass (Friedman and Halaas, 1998) and the hormone primarily acts to suppress appetite and increase energy expenditure (thermogenesis) (Houseknecht and Portocarrero 1998; Heshka and Jones, 2001). Consequently, circulating leptin levels fall during starvation and exhibit a diurnal pattern (Friedman and Halaas, 1998), with a pronounced nocturnal peak (Havel, 2001). Circulating levels of leptin show a circadian pattern in horses, with levels peaking at night and being lowest during daylight hours (Piccione et al., 2004; Buff et al., 2005). However, when horses are on pasture or are given ad libitum hay or frequent meals, circulating leptin levels to not fluctuate as greatly (Buff et al., 2005). Leptin is postulated to play an important role in energy balance and has been shown to have synergistic actions with cholecystokinin (CCK).

Adiponectin, a protein secreted by adipocytes, has been shown play a role in glucose and lipid metabolism (Berg et al., 2002) and energy homeostasis (Havel, 2001). Adiponectin decreases the postprandial rise of plasma free fatty acids and may be responsible for shifts in fuel utilization, favoring β-oxidation of fatty acids rather than carbohydrates as the primary energy source (Berg et al., 2002).

Hormones produced in the gastrointestinal tract, such as cholecystokinin, ghrelin, and peptide YY also play a role in the control of appetite. Cholecystokinin is a hormone produced by mucosal endocrine cells in the upper small intestine and is produced postprandially (Houpt, 1990; Small and Bloom, 2004). It has been shown to inhibit food intake in humans and rodents (Small and Bloom, 2004) and likely exerts a similar effect in the horse (Houpt, 1990). The role of cholecystokinin in regards to fat digestion has been discussed previously in this review. The release of CCK is primarily stimulated by dietary fat and by amino acids and small peptides released during protein digestion (Havel, 2001). Furthermore, because CCK is a potent inhibitor of gastric emptying, some of its effects to limit food intake may be indirectly mediated by the retention of food in the stomach (Havel, 2001).

Peptide YY, a peptide member of the NPY family, is produced by gut endocrine cells and released into circulation after meals (Adrian et al., 1985). It has been found to decrease appetite and inhibit food intake in the mouse, rat, and human (Batterham et al., 2002) and is suggested to be important in every-day regulation of food intake (Small and Bloom, 2004).

Ghrelin, a hormone synthesized in the stomach has been found to stimulate appetite in humans and rodents (Small and Bloom, 2004). Ghrelin levels are highest in the fasting state, rise sharply before a meal, and fall within one hour of a meal, or when nutrients are present in the stomach (Cummings et al., 2001; Havel, 2001). Ghrelin concentrations are lower in obese versus lean humans (Havel, 2001), indicating that it may play regulate day-to-day food intake (Small and Bloom, 2004).

Energy Balance

While hormones most definitely act to control appetite, a commonly held nutritional maxim is that horses, along with all species, eat to maintain their energy balance, or meet their energy requirements (NRC, 2007), so the number of calories ingested exceeds those expended (Houpt, 1990). Hoskin and Gee (2004) support this theory by suggesting that control of voluntary dry matter intake (VDMI) may be a function of DE intake. Thus, animals with previously insufficient or marginal DE intakes may show enhanced VDMI of high-quality pastures, whereas those with previously adequate DE intakes may reduce their VDMI when offered a pasture of higher DE content to maintain constant DE intakes (Hoskin and Gee, 2004). One study that supports this theory showed an increase in intake when ponies' feed was diluted with sawdust and a corresponding decrease in intake once the sawdust had been removed (Laut et al., 1985).

Patterns of seasonal feed intake plotted against weight gain or body weight (BW) have not totally upheld the concept that horses eat to maintain their energy balance (NRC, 2007). The body weight-time plots of pastured Przewalski's horses (Berger et al., 1999) showed increasing weight gains through spring, reaching a maximum weight in summer, followed by progressive weight loss through the autumn and winter. Weight gains through spring and summer averaged about 0.5 kg/d, followed by similar weight loss through autumn and winter (Berger et al., 1999; NRC, 2007). A high feeding rate coincided with low body weight in spring, followed by low feeding activity during peak body weights in summer with a return to high feeding activity in autumn and winter (Berger et al., 1999). The study did not

determine if weight loss in autumn and winter was due to poor quality feed, a reduction in voluntary feed intake, or cold weather extremes. The results of this study, therefore indicate that energy intake, and hence, BW, vary corresponding to a number of factors.

Body weight may partially be maintained by lipostatic mechanisms that regulate body fat stores. One theory of lipostatic control of body weight is that animals will eat to maintain their body weight when the available food is not very palatable but in the presence of palatable food, they will only gain weight to a certain point (Sclafani and Kluge, 1974). It is also theorized that there may be some glucostatic control of appetite. That is, when glucose levels fall below a certain level, far below normal, intake is stimulated (Houpt, 1990). The effect of glucose on control of appetite is discussed later in this review.

Physiological Mechanisms

Mechano- and chemoreceptors signaling the presence and energy density of food in the gastrointestinal tract contribute to satiety in the immediate postprandial period (Havel, 2001). Gastrointestinal mechano- and chemoreceptors have been found to respond to the products of digestion such as sugars, fatty acids, and amino acids (Havel, 2001). Furthermore, stretch and mechanoreceptors are activated by entry of food in to the stomach and proximal small intestine (Havel, 2001). In these ways, the physical and chemical properties of food can play a role in the short-term regulation of food intake through limiting the size of a single meal as well as residual effects on subsequent meals. A prime example of this is the decreased daily

food intake after consumption of a large volume meal with low nutrient and/or energy density.

Digestive Products

While all the effects of digestive products on hunger and satiety have not yet been elucidated, several theories exist relating digestive products to the control of hunger. However, there have been some conflicting reports of the effect of certain digestive products.

Digestion products in the intestine and VFA production in the cecum may be one mechanism contributing to the feeling of satiety or hunger (Frape, 2004). It is thought that when a certain concentration of these products (especially glucose) is met in the intestinal lumen and mucosa that eating stops, as mediated by vagal nerve fibers (Frape, 2004). When these concentrations have fallen below certain levels, eating recommences (Frape, 2004).

While volatile fatty acids (VFAs) (i.e. acetate, butyrate, propionate) are important contributors to the absorbed energy of the horse, especially with diets high in fiber, these compounds do not seem to have major effects on feeding behavior (Houpt, 1990). This is supported by Frape (2004) who suggests that VFAs have no sustained effect over 24 hours. However, it has been observed that in fasted ponies, an increase in gastric acetate tends to stimulate feeding, whereas relatively high cecal concentrations of VFAs, especially propionate, have an immediate but small depressing effect on appetite by extending the interval between meals and by reducing meal size (Ralston et al., 1983). However, there

were still no sustained effects over a 24-h period and reduced increases in cecal VFA may even stimulate appetite (Frape, 2004). While not discussed in this review, lactate, pyruvate, and ketones have also been found to inhibit feeding in animals (Havel, 2001).

Lipostatic control of appetite, or the regulation of BW by changes in body fat, as well as the effects of supplemental fat have also been suggested as a mechanisms contributing to both control of appetite and BW changes. Havel (2001) suggests that an increase of circulating lipids, in the absence of GI absorption, regulates feeding and that the rate of fatty acid oxidation may play a role in regulating food intake. This is supported by the findings of Houpt (1990) who postulates that while the administration of oil does not affect the onset of the first meal after a fast, it does prolong the feeling of satiety after the meal. The mechanism for this "fat-induced satiety" may be slower than that for "glucose-induced satiety" in part because of the slower speed at which fat leaves the stomach (Houpt, 1990). Furthermore, the presence of fat in the duodenum causes the release of the hormone cholecystokinin from the mucosal endocrine cells in the upper small intestine (Houpt, 1990; Small and Bloom, 2004). Cholecystokinin, as discussed previously, is released postprandially and has been shown to inhibit food intake in humans and rodents (Small and Bloom, 2004) and likely exerts a similar effect in the horse (Houpt, 1990). However, Frape (2004) reported that concentrations of NEFA in the blood are not significantly different between satiated animals and those with hunger. Despite the potential for fat and fat metabolism to inhibit food intake, there is also abundant evidence that consumption of diets

high in energy from fat leads to increased energy intake, BW gain, and obesity in humans and animals (Havel, 2001).

There is also some evidence that regulation of food intake may be mediated, in part, by dietary protein intake or increased circulating amino acids (Havel, 2001). Dietary protein has been found to induce satiety in the short term, and consumption of protein deficient diets leads to increased appetite for protein-containing foods. As such, amino acids may influence food intake either via direct actions within the central nervous system or via receptors located in the liver or portal vein (Havel, 2001).

Glucostatic control of appetite has been demonstrated in a wide variety of species, but insulin was not found to increase feed intake by horses in a study by (Ralston et al., 1979). However, it is clear that in humans and some animals, an acute lowering of circulating glucose concentrations will trigger sensations of hunger, and infusing glucose can decrease food intake (Havel, 2001). In horses, glucose administered intragastrically has also been shown to inhibit feed intake (Ralston et al., 1983). Furthermore, changes of glucose concentration or glucose utilization may also be involved in initiation of feeding (Havel, 2001). While animals with low concentrations of glucose available for the utilization in the brain tend to eat greater quantities more quickly, satiety in horses does not seem to be directly linked to changes in blood glucose (Frape, 2004). Furthermore, horses are more sensitive to increases in osmolality in the stomach caused by sodium chloride than they are to glucose (Ralston et al., 1983). It has also been suggested that the glucostatic and

glucodynamic models of food intake alone, are inadequate to explain the complex regulation of feeding behavior (Havel, 2001).

VDMI AND ASSOCIATED METHODS OF ESTIMATION

"The nutrient status of the herbivore depends on the nutritive value of the plants available, the botanical composition of the consumed diet, and the intake of the animal. … However, the nutrition of herbivores has always been hampered by difficulties in the estimation of their rate of nutrient intake (Dove and Mayes, 1996)." Although it may seem like a basic measurement, very little information is available on the voluntary dry matter intake (VDMI) of fresh forages by horses, and even less information is available on hourly DMI rate. This is partially due to the difficulties of measuring intake (NRC, 2007). Several methods have been used in horses and other grazing animals to estimate VDMI, though all methods have limitations. Methods include: subtraction of harvested residual herbage from calculated herbage mass allowance (Duren et al., 1989), the use of markers (Moffitt et al., 1987) such as n-alkanes (Nash and Thompson, 2001; Ordakowski et al., 2001), measurement of fecal outputs and known organic matter (OM) or DM digestibilities (Grace et al., 2002b; Grace et al., 2002a; Mesochina et al., 1998), "cut and carry" techniques to housed animals (Mesochina et al., 1998), and change in body weight (BW) after accounting for excretory outputs (Ince et al., 2005), or through determination of bite size, number, and duration of feedings (Duren et al., 1989). The use of these methods has produced a wide range of hourly and daily DMI estimates for horses of varying characteristics. The NRC (2007) suggests that daily pasture intakes ranged from 0.015 - 0.032 kg DM · kg BW^{-1} · d^{-1} (average 0.020 kg DM · kg BW^{-1} ·

d^{-1}). A number of experiments estimating DMI and the associated methods are discussed here.

HOURLY ESTIMATES

Change in BW

Ince et al. (2005) used 3 Welsh-cross pony geldings (mean BW 338.5 ± 100.70 kg) to determine intake over a 3-hr period on grass pasture. The ponies were stabled for 21 hr/day and were fed meadow hay at 0.010 kg DM · kg LW^{-1} · d^{-1} and were permitted to graze grass pasture (13% DM, 26.5 cm sward length) for the remaining 3 hours of the day. Forage DMI over the 3-h grazing period was estimated by measuring live weight (LW) change over the grazing period. This method involved weighing the ponies prior to and after the 3-h grazing period and collecting any urine and feces during the grazing period. Forage intake was considered to be the difference in pre- and post-grazing BW, after correcting for fecal and urinary losses. Ince et al. (2005) found that the ponies consumed 21% of their daily DMI during the 3 hr on pasture. With an average of 15 hours per day grazing, this consumption may suggest a steady intake of DM over the grazing period (Ince et al., 2005) as the ponies consumed about 1/5 of their requirement in approximately 1/5 of the amount of time they would graze each day. The mean DMI rate per hour for this group of ponies was 0.087 kgDM · 100 $kgBW^{-1}$ · h^{-1}, (range 0.057 to 0.136 kgDM · 100 $kgBW^{-1}$ · h^{-1}) which corresponds to an intake of 0.17 to 0.4 % BW (mean; 0.26% BW) over 3 hours or, when extrapolated to 15 hours of grazing time, corresponds to 0.85 to 2.0% of BW (mean; 1.3% of BW). This seems to be a reasonable estimate when considering the ponies were being fed supplemental hay. However, it must also be noted that the forage grazed in this study had

relatively high moisture content (87% moisture) in comparison to those typically available (mean 49%; range 20 – 80% moisture) (Dairy-One forage database, Ithaca, NY).

While this study yielded reasonable estimates of herbage intake, there are drawbacks to the method of estimation. In order to be accurate, the method of estimating herbage intake by changes in BW requires very precise measurements of BW. This may be one benefit of the use of ponies in the study by Ince et al. (2005), as they are typically smaller and have less BW than horses. Penning and Hooper (1985) successfully used this method in 1985 to estimate herbage intake in sheep grazing pasture. Therefore, this method may lend itself better to trials with smaller animals where more precise scales can be used. The scale used by Penning and Hooper (1985) had a range of 0 to 240 kg ± 10g and therefore was able to detect very small changes in BW of the sheep. With less BW, it is likely easier to access a scale where BW can be measured precisely, whereas with larger animals, access to a scale with this ability could be limiting. Furthermore, in order to estimate intake using this method, one must also consider weight- loss not associated with feces and/or urine, such as evaporative losses, saliva, and sweat, which are commonly referred to as insensible losses. While these losses are in most cases small, they still represent error within the calculation. However, the advantages of this method include ease of use, ability to measure intake over short periods of time, and ability to account for changes in forage conditions (Penning and Hooper, 1985).

Residual Herbage Mass Method

The hourly DMI rate estimated by Ince et al. (2005) is only about half that estimated by Duren et al. (1989), using a residual herbage mass method. Duren estimated voluntary herbage dry matter intake of Thoroughbred and Quarter Horse yearlings (6 colts, 2 fillies; mean BW 374 kg) grazing Orchardgrass pasture (sward height 24 cm) during a 3-h grazing session. To measure intake, ungrazed, control strips (17.5 x 1.5 m) were harvested to 5 cm height with a Haldrup forage harvester. Horses then grazed within a defined area for 3 hr after which time, the residual herbage above 5 cm, within the area, was harvested (Duren et al., 1989). Dry matter intake was estimated from the difference between herbage mass allowance and residual herbage dry matter in the grazing area. Cantillon, (1986) had previously established biomass collection as a viable means of estimating forage intake in horses by simultaneously comparing biomass collection to an internal marker technique for determining intake.

Duren et al. (1989) determined the mean intake of a group of Thoroughbred yearlings to be 0.62 kg DM/h, which corresponds to 0.164 kg DM $\cdot$ 100 kg $BW^{-1} \cdot h^{-1}$. Extrapolated to 15 hours of grazing per day, this corresponds to 9.2 kg DM/day which is a DMI of 2.46% BW/day. While this estimate is greater than that of Ince et al. (2005) it is still within the range suggested by NRC (2007), and seems to be reasonable. The increased DMI rate in the yearlings Duren et al. (1989) compared to that of the ponies could likely be attributed to differences in physiological state of the animals, forage characteristics, management (e.g. exercise), and supplemental feed. While Duren et al. (1989) used growing horses of light

breeds, some of which were being exercised, Ince et al. (2005) used ponies (age/physiological state not reported) that were not exercised. Furthermore, while sward height was similar in both studies, DM percentage of the forage grazed in Ince et al. (2005) was only half of that grazed in Duren's study (13 vs. 26% DM, respectively). This and other differences in forage composition could have affected intake, along with differences in supplemental feed, either in the form of hay (Ince et al., 2005) or hay and grain (Duren et al., 1989).

In a separate, but similar, study, yearling horses grazing endophyte-free tall fescue had a forage intake of 3.2 kg DM during a 3-h grazing session (Duren et al., 1987). This intake corresponds to 1.07 kg DM/h, and assuming the yearlings had a BW of about 350kg (70% of a mature weight of 500kg), corresponds to an intake of 0.31 kg DM · 100 kg BW^{-1} · h^{-1} or a daily intake (assuming 15 hours of grazing) of 16.28 kg DM/day (4.65 % BW). Cantillon (1986) used a similar technique and found mature horses to have forage DM intakes of 1.5 and 1.65 kg DM/hr while grazing endophyte-free tall fescue and alfalfa, respectively (Duren et al., 1989). Assuming the mature horses had a BW of 500kg, this corresponds to an intake of 0.30 kg DM · 100 kg BW^{-1} · h^{-1} and 0.33 kg DM · 100 kg BW^{-1} · h^{-1} for the endophyte-free tall fescue and alfalfa, respectively. Again, assuming a 15 hours of grazing per day, these estimates can be extrapolated to 22.5 kg DM/day (4.5% BW/day) for endophyte-free tall fescue and 24.75 kg DM/day (5.0% BW/day) for alfalfa. These estimates are much higher than the estimates presented by the NRC (2007), and seem unlikely for horses at maintenance. Assuming yearlings weigh 70% as much as the mature horses (Lewis, 1995),

these DMI estimates are nearly identical on a body-weight basis. However, the method of estimation and other details of these studies (i.e. management of horses, supplemental feed, forage characteristics) are not available.

DAILY ESTIMATES

While few estimates are available for hourly DMI, there have been a number of estimates of daily DMI using a variety of methods, animals, and forages.

Forage Digestibility and Fecal Mass Method

Mesochina et al. (2000) estimated grass intake of growing Selle Francais horses (yearlings and two-year olds) grazing grass pasture of three different sward heights (6.6, 8.1, and 9.4 cm). They estimated pasture intake using the following equation: Intake = weight of feces produced daily/(1 - forage digestibility) and found the mean intake to be 0.082 kg OM $\cdot$ kg^{-1} LW$^{0.75}$ $\cdot$ d^{-1}. They also found that pasture intake based on metabolic live weight did not differ between yearlings and 2-year-olds, which is in agreement with the findings of (Cantillon, 1986; Duren et al., 1989). However, they did determine that the rate of DMI of the 2-year-olds was greater than that of the yearlings (0.0099 vs. 0.0076 kg OM $\cdot$ min^{-1}) (Mesochina et al., 2000). They also found that while sward height did not affect time spent grazing or DMI, there was a significant effect ($P < 0.01$) of age, with the yearlings spending 65.8 % of their time grazing whereas the 2-year-olds only spent 58.8% of their time grazing. The forage used in this trial had a DM content ranging from 21.2 – 30.3%, a CP content ranging from 17.0 – 21.8%, and a NDF of 62.4 – 64.4%. In this trial, it is possible that the

difference in sward height (6.6 – 9.1cm) did not vary enough to elicit a response in the horses.

Fleurance et al., (2001) measured daily forage intake of heavy breed mares grazing heterogeneous wet grassland in France during the summer using a very similar method. They estimated daily DMI using the following equation: organic matter intake = weight of feces produced over 24 hr / (1- organic matter digestibility), where organic matter digestibility was estimated by the crude protein content of the feces (Fleurance et al., 2001). The results of this study estimated mean intake to be $.173 \pm .023$ kg DM $\cdot$ kg $LW^{0.75} \cdot d^{-1}$, which is higher than most other estimates. These mares also spent much of their time grazing short grass lawns, as was discussed earlier in this review. The forage grazed in this trial had a CP content of 8.4 – 14.1%, and an NDF of 58.9 – 72.6%. While these DMI estimates are greater than most other estimates, the small number of animals and the potential breed effect of using heavy-breed animals should be taken into consideration.

In a series of similar experiments conducted in New Zealand, Grace et al. (2002a) estimated the mean daily DMI and DEI of different groups of horses grazing ryegrass/white clover pastures. They estimated daily DMI by: DMI = daily fecal DM output / (1- digestible DM) (Grace et al., 2002a). Digestible energy intake (DEI) was determined from the difference between the gross energy intake and the gross energy fecal output. They found the DMI of yearling horses grazing the ryegrass/white clover pasture in mid to late spring to be 6.9 ± 0.3 kg DM/d. The mean BW of the yearlings was 350 kg, therefore, when scaled to BW, this

DMI corresponds to 1.97 kg DM · 100 kg BW· d^{-1} or assuming 15 hours of grazing, an hourly estimate of 0.13 kg DM · 100 kg BW· h^{-1}. They found the DEI to be 78 ± 2.9 MJ DE/d which corresponds to 22.29 MJ DE · 100 kg BW· d^{-1}, on a BW basis. The forage grazed in this experiment had the following characteristics: CP (14.0 – 15.5%), NDF (43.2 – 43.8%), ADF (24.2 – 27.5%), lipid (2.85 – 2.9%), ash (10.5 – 10.7%), and soluble carbohydrate (7.1 – 8.5%).

Grace et al. (2002b) used the same method to estimate the DMI and DEI of pasture-fed lactating Thoroughbred mares grazing ryegrass/white clover pasture in late spring. They found the mean DMI to be 13.6 ± 0.8 kg DM/d, or assuming 15 hr of grazing, (0.162 kg DM · 100 kg BW· h^{-1}) with a corresponding DEI of 146.9 ± 8.4 MJ DE/d. The mean BW of the mares was 560 kg, so when scaled to BW the DMI and DEI are 2.43 kg DM · 100 kg BW· d^{-1} and 26.2 MJ DE · 100 kg BW· d^{-1}, respectively. The forage grazed in this experiment had the following characteristics: CP (18.2 – 19.8%), NDF (48.5 – 50.3%), ADF (30.4 – 32.4%), lipid (2.4 – 2.6%), and soluble carbohydrate (10.5 – 11.9%).

Grace et al. (2003) also used this method to estimate daily DMI and DEI of weanlings grazing perennial ryegrass/white clover pasture in the fall. They estimated the DMI and DEI to be 5.5 ± 0.26 kg DM/day and 62.7 ± 2.75 MJ DE/day, respectively (Grace et al., 2003). The mean BW of the weanlings was 300kg, so the DMI and DEI scaled to BW are 1.83 kg DM · 100 kg BW· d^{-1} and 20.9 MJ DE · 100 kg BW· d^{-1}, respectively. Assuming 15 hr of grazing time, the DMI estimate corresponds to an hourly estimate of 0.12 kg DM · 100 kg

$BW \cdot h^{-1}$. The pasture grazed in this study had the following characteristics: CP (22.2 ± 0.5%), NDF (7.2 ± 0.4%), ADF (23.9 ± 1.1%), lipid (3.3 ± 0.2%), and soluble carbohydrate (7.2 ± 0.4%).

Digestibility Markers

One common method used to estimate the herbage intake of grazing animals is the use of external and/or internal markers. Using this method, the naturally occurring indicators present in plants serve as an index of indigestibility when measured in the feces (Smith and Reid, 1955). A separate indicator can be administered to an animal in a known and constant amount over time and can provide a measure of the total fecal output (Smith and Reid, 1955). The forage dry matter intake can then be calculated using the indigestibility estimate along with the fecal output (Smith and Reid, 1955). The use of Cr_2O_3 (Chromic Oxide) as an indicator of fecal output and plant chromagen or nitrogen as an indicator of indigestibility yielded accurate estimates of intake in grazing cows (Smith and Reid, 1955). However, possible errors involved with estimating fecal output from daily or more frequent dosing with Cr_2O_3 have been detected (Dove and Mayes, 1996). In equine studies, acid insoluble ash (AIA) has been the most frequently used internal marker, but it does present difficulties in analysis (Ordakowski et al., 2001).

Moffitt et al. (1987) used a marker technique to estimate the DMI of 2-year-old horses in different seasons grazing either orchardgrass/clover or fescue pasture. They used a double marker technique with indigestible neutral detergent (INDF) and Ytterbium (Yb) as internal

and external markers, respectively. They found the DMI of fescue pasture to be 6.79, 10.90, and 10.31 kg DM/d in December, May, and August, respectively, with the DMI in December being significantly less than that in May and August. Assuming a mean BW of 429 kg for 2-year-old horses NRC (2007) and a 15 hr grazing time, these estimates correspond to intakes of (0.11, 0.17, and .16 kg DM · 100 kg BW^{-1} · h^{-1}) or (1.58, 2.54, and 2.40% BW/d) for December, May, and August, respectively. The DMI of orchardgrass/clover pasture was determined to be 8.24, 8.67, and 12.77 kg DM/d in December, May, and August, respectively, with the DMI in August being significantly greater than that in December or May. Again, assuming a mean BW of 429 kg, these estimates correspond to intakes of 1.92, 2.02, and 2.98.65% BW/d for December, May, and August, respectively. While all of these estimates fall within the range suggested by the NRC (2007) and seem reasonable, it must be noted that a mean BW of 429 kg is only an estimate and most likely would have varied from December to August. While it was determined that DM, ADF, and NDF digestibilities were greater for orchardgrass than fescue during the summer, the DMI was not different between forages (Moffitt et al., 1987). They did determine that Orchardgrass/clover DMI was lowest in the winter (December) ($P < 0.05$) while fescue DMI was greatest in the summer (August) ($P < 0.05$). The characteristics of both the Orchardgrass and fescue pastures varied by season. The fescue pasture had the following range in characteristics, DM (25.8 – 39.6%), CP (12.2 – 23.2%), ADF (22.8 – 38.9%), and NDF (69.0 – 80.6%). The Orchardgrass pasture had the following range in characteristics, DM (19.0 – 34.3%), CP (15.8 – 19.5%), ADF (31.5 – 44.1%), and NDF (64.4 – 80.3%). Overall, NDF was lowest in the spring and highest in the winter and both pastures tended to be most digestible during the summer. Dry

matter intake was lower ($P < 0.05$) in winter than in summer, but no differences were found in forage component digestibilities or DM intake among horses within trials and forages. They also determined that while Ytterbium appeared to be an effective external marker to determine fecal output, INDF may not be the most reliable internal marker for use in horses.

Alkanes

An alternative method based on the analysis of plant alkanes in herbage and feces has been used to estimate DMI in ruminants and horses (Dove and Mayes, 1996; Ordakowski et al., 2001). Plant alkanes are predominantly indigestible, odd-chain hydrocarbons, found in the plant cuticular wax (Dove and Mayes, 1996). The n-alkanes are saturated straight-chain hydrocarbons (Ordakowski et al., 2001). Plant alkanes can be used, in combination with orally dosed even-chain alkanes, to obtain an intake estimate that is essentially independent of marker recovery in feces (Dove and Mayes, 1996). A double alkane procedure has been used for estimating intake by dosing animals with known quantities of an even-chain alkane. Intake is estimated from the daily dose rate and the dietary and fecal concentrations of the dosed, even-chain alkane and a natural, odd-chain alkane adjacent in chain length. Intake is computed using the following equation: Intake = $(F_i/F_j) * D_j / [H_i – (F_i/F_j)H_j]$, where H_i and F_i are the herbage and fecal concentrations of the odd-chain alkane; H_j and F_j are the equivalent concentrations of the even-chain, dosed alkane (of which there will be a small amount in herbage), and D_j is the daily dose of the even-chain alkane (Dove and Mayes, 1996). This method has been shown to provide accurate estimates of intake and it also has other advantages including; allowing for between animal variation in diet digestibility,

determination of diet digestibility when dosed concurrently with an external marker, accommodating for the feeding of supplements, use in obtaining individual intakes in housed animals, and for determination of intake of individual plant species or parts (Dove and Mayes, 1996). Furthermore, using n-alkanes as internal markers has the advantages of decreased costs and ease of preparation and analysis (Ordakowski et al., 2001). Dove and Mayes (1996) suggest that estimates of intake obtained with alkanes appear to be more accurate than those obtained with previous methods and also that n-alkanes can be used to accurately estimate intakes in free ranging herbivores (Dove and Mayes, 1996). However, Ordakowski et al. (2001) found that horses are capable of metabolizing alkanes to some extent and therefore to use plant alkanes as internal markers to obtain accurate estimates of dry matter digestibility, it may be necessary to adjust initial fecal alkane concentrations (Ordakowski et al., 2001).

Friend and Nash (2000) also estimated organic matter intake (OMI) of weanlings grazing improved or unimproved grass pasture at high and low stocking rates across seasons (November, May, August, and October) using the n-alkane method. They dosed the weanlings with 500mg of C_{32} and then collected pasture and fecal samples to measure for individual alkane content. Intake was calculated for each weanling according to the equation of (Dove and Mayes, 1996) (shown above). They found that total intake varied significantly between treatment groups (improved low, improved high, unimproved low, unimproved high). On improved pastures, intake was greater at the low stocking rate than high Friend and Nash (2000). Comparing pasture types, at the low stocking rate, intake of improved

pasture was greater than unimproved, while at the high stocking rate, intake of the unimproved pasture was greater than that of the improved (Friend and Nash, 2000). Furthermore, it was found that intake was greatest during October, with decreased intakes in August, November and May. All of the seasonal intakes differed from each other except between November and May. On improved pasture the intake was greater at the low stocking rate. The overall treatment effect was that weanlings grazing improved pasture at a low stocking rate grazed at a greater rate than those grazing improved pasture at a high stocking rate. Conversely, for unimproved pastures, intake per unit BW tended to be greater at the high stocking rate. In this case, it is likely that intake was more limited by herbage mass than quality. Overall, Friend and Nash (2000) found that in order to maximize intake of weanlings, it is more important to increase grazing pressure on unimproved pastures, while on improved pastures a lower stocking rate may increase intake. Moreover, there appeared to be no benefit, in terms of intake, of grazing improved pasture, relative to unimproved pasture. However, the relatively high quality and adequate mass of the unimproved pastures in this study must be taken into consideration.

Friend and Nash (2000) estimated these weanlings consumed between about 4.2 and 12.2 kg OM/day in November, about 2.6 and 3.9 kg OM/day in May, about 10.2 to 22 kg OM/day in August, and about 19 to 42 kg OM/day in October. When scaled to a BW basis (assuming 257 kg BW for an 8 month old weanling; NRC, 2007), these intakes correspond to a daily DMI of between 1.60 and 4.75 kg DM · 100 kg BW· d^{-1} in November, 1.01 and 1.52 kg DM · 100 kg BW· d^{-1} in May, 3.97 and 8.56 kg DM · 100 kg BW· d^{-1} in August, and 7.40 and 16.34

kg DM · 100 kg BW· d^{-1} in October. Assuming a grazing time of 15 hr, the estimates correspond to estimates of between 0.11 and 0.32 kg DM · 100 kg BW· h^{-1} in November, 0.07 and 0.10 kg DM · 100 kg BW· d^{-1} in May, 0.26 and 0.57 kg DM · 100 kg BW· d^{-1} in August, and 0.49 and 1.09 kg DM · 100 kg BW· d^{-1} in October. The organic matter digestibility of the forage utilized in this study is as follows: in August digestibility ranged from about 62 to 78% whereas in October, digestibility ranged from about 45 to 70%. Digestibility of forage was not determined for November or May.

Intake per unit of bodyweight in these weanlings was greater during October than August, which was in turn, greater than the intake per unit bodyweight in May or November (Friend and Nash, 2000). These estimates of intake during August and October are unusually high and are far greater than those estimated by the (NRC, 2007). One potential reason for this heightened estimate in October was that the pasture had gone to seed and it was reported to be difficult to obtain pasture samples that accurately represented what the horses were consuming. This could have caused an overestimation of pasture intake (Friend and Nash, 2000). Therefore, it is suggested that when using the n-alkane method to estimate intake of horses grazing mature pastures or rangeland, that analysis of individual plant species and components may be required to obtain reliable estimates of intake (Friend and Nash, 2000). It is also possible that the dose of C_{32} was less than intended and that a more reliable dosing technique is required.

McMeniman (2003) also conducted an experiment in New Zealand to investigate effect of changing pasture characteristics on intake of stock horse yearlings. He found the mean DMI of yearlings grazing mixed blue grass, lucerne pasture to be 13.3 ± 3.54 kg DM/d (3.3 ± 0.99% BW/d), 8.4 ± 5.52 kg DM/d (2.9 ± 1.61% BW/d), and 9.8 ± 3.82 kg DM/d (2.9 ± 0.96% BW/d), for mean sward heights of 11, 23, and 47cm, respectively. In a different component of the trial, he found the mean DMI of yearlings grazing mixed blue grass, African star grass, and lucerne pasture to be 7.1 ± 1.31 kg DM/d (1.8 ± 0.39% BW/d), 9.1 ± 1.92 kg DM/d (2.4 ± 0.45% BW/d), and 6.8 ± 3.67 kg DM/d (1.8 ± 1.04% BW/d), for mean sward heights of 6, 16, and 25 cm, respectively. In a third aspect of the trial, it was determined that the mean DMI of yearlings grazing pastures of differing HM density was 6.3 ± 1.35 kg DM/d (1.5 ± 0.43% BW/d), 6.0 ± 1.47 kg DM/d (1.5 ± 0.32% BW/d), and 6.8 ± 3.11 kg DM/d (1.6 ± 0.64% BW/d), for least dense to most dense pastures. Overall, the DMI of the horses in the experiments, estimated using the alkane marker technique, ranged from 1.5 to 3.3% of live weight. These estimates are nearly identical to those of the (NRC, 2007). The horses exhibited a high level of diet selectivity that suggests a relationship between the percentage leaf in the diet and DMI. The results also indicate a relationship between dry matter intake and the digestibility of the diet eaten. Furthermore, McMeniman (2003) concluded that if horses have access to pastures with a high dry matter digestibility, their DMI could be optimized.

Cut & Carry Technique

Dulphy et al. (1997) used the "cut & carry" technique to estimate VDMI by trough feeding forage to a group of light horse geldings at maintenance. This "cut & carry," method is so named because fresh forage is harvested and provided to horses in stalls. Dulphy et al. (1997) found the VDMI of 1st cycle fresh forages to be 0.087 kg/ kg $LW^{0.75}$ (range 0.077 – 0.091 kg/ kg $LW^{0.75}$) whereas the VDMI of 2nd cycle fresh forages was significantly different ($P < 0.05$) at 0.096 kg/ kg $LW^{0.75}$ (range 0.078 – 0.123 kg/ kg $LW^{0.75}$). Only the intake of the 1st cycle fresh forages in Dulphy et al. (1997) was significantly different from the estimate of .105 kg/kg $LW^{0.75}$ (range 0.090 – 0.117 kg/ kg $LW^{0.75}$) found in the literature. The forage characteristics were as follows: 1st cycle fresh forage – CP 19.4% (range 13.9 – 29.1%), crude fiber 23.2% (range 15.0 – 30.3%), and NDF 50.8% (range 30.0 – 61.1%); 2nd cycle fresh forage – CP 14.9% (range 11.8 – 20.0%), crude fiber 25.6% (range 24.0 – 27.0%), and NDF 57.7% (range 53.5 – 60.0%). The intake (after correction for live weight) was not influenced by CP, crude fiber, or NDF. However, it is postulated that the organoleptic qualities of forages (taste, color, odor, ease of prehension, toughness, ease of sorting etc.) are likely important factors influencing intake in horses as they are in other species (Dulphy et al., 1997). Therefore, by manually harvesting the forage and then providing it the horses, there may be aspects of grazing behavior and VDMI that are overlooked.

Chenost and Martin-Rosset (1985) also used the "cut & carry" technique to compare the intake and digestibility of forages between cows, sheep, and horses. They found the mean DMI of the horses to be 0.0986 kg DM/kg $W^{0.75}$ (range 0.0774 - .123 kg DM/kg $W^{0.75}$).

Shingu et al. (2000) conducted a trial in Japan aimed at estimating VDMI of Hokkaido native horses (dry and lactating mares) and light half-breed horses (dry mares) on woodland pasture. The VDMI of the Hokkaido native horses was 0.088 kg DM/kg metabolic body weight (MBW) whereas the VDMI of the light half-bred horses was 0.086 kg DM/kg MBW. Both groups of horses lost weight over the experimental period (23 days), with the light horses losing 0.82 ± 0.2 kg/day and the Hokkaido native horses losing 0.50 ± 0.5 kg/day.

In summary, while estimates of hourly DMI and daily DMI for horses grazing pasture, or being provided fresh forages, are present in the literature, validation of these estimates is not often present. As such, it is difficult to understand if the current estimates are valid. Furthermore, the estimates vary widely depending on a number of factors, including forage characteristics, physiological state, and environmental variables.

CHAPTER 2

PREDICTION OF HOURLY PASTURE DRY MATTER INTAKE IN HORSES

Introduction

Determining pasture's nutrient contribution toward total daily nutrient requirements depends upon an estimate of pasture dry matter intake (DMI). Measurements of pasture DMI and subsequent prediction equations for horses have been reported for 24-hr grazing periods (NRC, 2007), as well as shorter 3-hr periods (Ince et al., 2005; Duren et al., 1987 and 1989). The validity of these estimates when applied to intermediate grazing periods (i.e., greater than 3 hr but less than 24 hr) has not been established and relies on the assumption that the rate of pasture DMI remains constant, which may not be true due to changing environmental and physiological factors that introduce variability in pasture DMI throughout the day (NRC, 2007). Additionally, changes in seasonal characteristics of pasture forage (e.g. available herbage mass, stage of plant maturity, etc) may also influence hourly intake (reference from literature review). Therefore two experiments were conducted to obtain and validate estimates of hourly pasture DMI rates over an 8-hr period in three different seasons of the year.

Materials and Methods

The following experimental protocol was approved by the North Carolina State University Animal Care and Use Committee (Protocol 08-084-A).

Experiment 1

Eight horses (7 mares, 1 gelding) of light horse breeding ranging in age from 8 to 25 yr of age (mean ± SD; 15.6 ± 6.8) and weighing 576 ± 32 kg (mean ± SD) were used to determine pasture DMI over an 8-h period in October 2008, and February 2009, and May 2009. Each of these seasons is henceforth referred to as period 1, 2, and 3 respectively. Individual characteristics (e.g., breed/type, age, gender) of each horse are shown in Table 1. Each horse was randomly assigned to one of eight adjacent 17 m x 34 m enclosures (2 rows of 4 adjacent enclosures constructed with 1.27 cm poly tape electric fencing) for a 5 to10-d adaptation period prior to the experiment's start. During the adaptation period, horses were acclimated to grazing individually and to the 1.27 cm poly tape electrical fencing. Enclosures used during the adaptation period contained tall fescue (MaxQ®, Pennington Seed, Madison, GA). Within each enclosure were two adjacent 5 m x 5 m cells (cell A and cell B) that were used to measure DMI in two consecutive 4 h periods. Henceforth, these cells are referred to as DMI cells A and B. Each of the 5 m^2 cells was constructed of 2 strands of 1.27 cm poly tape and contained the same tall fescue. The 5 m x 5 m cell size was determined, based on observations from a preliminary experiment, to be small enough to accurately estimate forage yield, by means of measuring herbage mass reduction, and large enough to prevent excessive trampling and ensure forage was not limiting during each 4-h period. A diagram of the 5 m^2

cells in relation to the adaptation cells is shown in Figure 1. The DMI cells A and B were located on the 34 m side of each enclosure (see Figure 1). The chemical composition of the tall fescue in each cell A and B is shown in Table 2.

A timeline showing the design of experiment 1 (EXPT1) can be seen in Figure 2. Prior to measuring DMI all horses were muzzled (standard horse grazing muzzle, Best Friend Equine Supply, New Holland, PA) for approximately 12 h. Horses were muzzled to prevent possible residual effects from differences in DMI immediately prior to commencement of the experiment as well as to ensure horses would graze within the cell. Muzzles were constructed of lightweight nylon and rubber with a 2.54 cm opening at the bottom of the muzzle to allow for water consumption. Horses fitted with muzzles may have had some DMI, but it was assumed to be minimal. On the day prior to the experiment and the day of the experiment, horses were weighed at 700 h using a digital livestock scale with ±1.0 kg sensitivity (Smart Scale 200, Gallagher Animal Management, USA). On the day of the experiment, horses were allowed to graze their first cell (cell A) for 4 h beginning at approximately 800 h. At the end of 4 h, horses were moved immediately to the second cell (cell B) for an additional 4 h of grazing. Pasture intake over each 4-h period was estimated by subtracting residual herbage mass (HM) following 4 h of grazing from the initial HM and was then expressed as DM · 100 kg BW^{-1} · h^{-1}. Initial and residual HM were measured by harvesting and weighing four randomly selected 1 m^2 subplots within each 5 m^2 DMI cell. Forage was harvested to below observed grazing height (approximately 3 cm above ground level), collected, weighed, and a composite sample stored at -20 °C. Forage was harvested

using either manual hedge trimmers or gas powered hedge trimmers. Chemical composition was analyzed on all forage samples by the North Carolina Department of Agriculture Forage Testing Laboratory (Raleigh, NC). Forage samples were also submitted to the Dairy One Forage Laboratory (Ithaca, NY) for analysis of ethanol soluble carbohydrates (ESC), water-soluble carbohydrates (WSC), and starch. Forage digestible energy (DE) concentration was calculated using chemical composition results according to the NRC (2007).

All eight horses had been maintained in the same facility under similar conditions for at least 12 months prior to the experiment. Horses had access to water at all times during the experiment.

Differences in pasture DMI rates and forage chemical composition of the grazing cells during the first and second 4-h periods across periods were analyzed as a repeated measures design using Proc Mixed of SAS, version 9.1 (SAS, Cary, NC) according to (Littell et al., 1998). The model included cell (cell A and cell B), period (1, 2, 3), and their interaction. The model subject was horse, and repeated time was (cell x period). The most appropriate covariate structure was determined by selecting the structure that yielded the lowest Akaike's Information Criterion, Schwarz's Bayesian Criterion, and -2 Res Log Likelihood values. The mean value of each chemical analysis component, calculated from the pre- and post-grazing samples, was used to represent cell A and cell B in the above statistical analysis. A *P*-value of < 0.05 was considered significant. When main effects (cell, period or their interaction) were significant, means were separated using a t-test with the Tukey-Kramer adjustment.

Results are presented as means ± SE. Results of experiment 1 (EXPT1) - period 1 are based on 7 observations as data from one horse were compromised and therefore not included.

Experiment 2

To determine the validity of hourly DMI estimated in EXPT1, the same 8 horses used in EXPT1 were randomly assigned to one of two treatments: unrestricted (UNRES; n = 4) or restricted (RES; n = 4) grazing. Unrestricted horses had access to pasture at all times, whereas RES horses had a grazing time that was restricted to a period calculated to provide intake of only the daily maintenance DE required. Validity of hourly DMI was assessed by monitoring energy balance, measured by changes in body weight over a 42 d period in the RES treatment and comparing them to changes in the UNRES treatment over the same period. Grazing time for horses in the RES treatment was calculated as the time necessary to consume maintenance DE requirements based on: average pasture DMI rate from EXPT1, pasture DE concentration (calculated using chemical composition measures according to NRC, 2007), and horse BW. Grazing times were updated regularly based on changes in forage DE concentration. Therefore, if the hourly DMI rate determined in EXPT1 was correct, RES horses should have little or no changes in BW. Experiment 2 (EXPT2) began approximately one week after EXPT1 all three periods (1, 2, 3). Experiment 2 beginning in October and concluding in December is hereafter referred to as period 1, EXPT 2 beginning in February and concluding in April is hereafter referred to as period 2, and EXPT 2 beginning in May and concluding in July is hereafter referred to as period 3. A summary of the details of EXPT2 for each period can be found in Table 4.

Using the average DMI rate from EXPT1, pasture DE estimates and each horses' maintenance DE requirement, the time calculated for the RES horses to graze was 9 h during period 1, 17 to 19 h during period 2, and 14 to 15 h during period 3. The UNRES horses had continuous access to pasture over each 42-d period. Grazing was restricted by use of the same muzzles used in EXPT1. Muzzles were removed each morning at 800 h and replaced at 1700 h during period 1. During period 2, muzzles were removed each evening at 1630 h and were replaced at 1130 h the following day. During period 3, muzzles were removed each evening at 1800 h and were replaced each morning at 800 or 900 h.

Two pastures, (pasture 1 and pasture 2) containing tall fescue similar to that in EXPT1 were used for the 42-d validation period (Figure 3), except in period 2, where three pastures (pasture 1, 2, and 3) were used for a 35-d validation period, due to limiting forage in each pasture. In period 1 and 3, pastures 1 and 2 were divided into eight 25m x 33m enclosures (two rows of four adjacent enclosures) and were grazed for 21 d. In period 2, pasture 1 was divided into eight 17m x 34m enclosures, and pastures 2 and 3 were the same as in periods 1 and 3. Each horse was randomly assigned to an enclosure within each pasture. Horses grazed the enclosures of pasture 1 for the first 21 d and the enclosures of pasture 2 for the second and consecutive 21 d in periods 1 and 3. In period 2, pasture 1 was grazed for 13 d, while pastures 2 and 3 were each grazed for 11 d. The mean chemical composition of the tall fescue in each pasture of period 1, 2, and 3 is shown in Table 5, 6, and 7, respectively.

Figure 4 shows the measurements taken during EXPT2 and the times at which they were collected. Body weight was monitored weekly between 700 and 800 h with an electronic scale (sensitivity = ± 1 kg; Smart Scale 200, Gallagher Animal Management, USA). Forage from four randomly selected $1m^2$ subplots within each enclosure was harvested, as described in EXPT1, prior to and immediately after horses grazed each pasture. Proximate analysis, as described in EXPT1, was performed on a composite sample obtained from each enclosure. Forage DE concentration was then calculated using proximate analysis measures according to the NRC (2007). Initial and residual HM of each enclosure within pastures 1, 2, and 3 were calculated as in EXPT1.

Response variables (BW, initial HM DM prior to grazing, HM DM difference, forage chemical composition) within periods 1, 2, and 3 were analyzed as a repeated measures design using Proc Mixed of SAS, version 9.1 (SAS, Cary, NC) according to (Littell et al., 1998). The model included treatment (RES vs. UNRES), sampling time, and their interaction. The model subject was horse within a treatment. The most appropriate covariate structure was determined by selecting the structure that yielded the lowest Akaike's Information Criterion, Schwarz's Bayesian Criterion, and -2 Res Log Likelihood values. Horse BW was analyzed over the entire 42 d (35 d in period 2); therefore week (i.e. weekly BW measurement) was the repeated time in the model. Herbage mass DM difference in each horse's pasture was represented by the difference between the pre- and post-grazing HM DM, and therefore pasture was considered as the repeated time in the model. Forage chemical composition values are represented as an average of the initial pre-grazing and final

post-grazing values for each individual horse's pasture. Therefore, pasture is considered the repeated time in the model. A level of $P < 0.05$ was considered significant. When main effects (time, treatment, and their interaction) were significant, means were separated using a t-test with the Tukey-Kramer adjustment. Results are presented as means ± SE.

Results

Experiment 1

The average hourly pasture DMI for cells A and B, within each period are shown in Table 3. The average hourly pasture DMI rate during the 8-h grazing period was 0.166, 0.088, and 0.108 ± 0.013 kg DM · 100 kg BW^{-1} · h^{-1} in periods 1, 2, and 3, respectively (Figure 5). There were significant differences in the 8-h hourly DMI between periods ($P = 0.001$). The 8-h hourly DMI in Period 1 (0.166 ± 0.013 kg DM · 100 kg BW^{-1} · h^{-1}) was greater ($P < 0.001$) than that of Period 2 (0.088 ± 0.013 kg DM · 100 kg BW^{-1} · h^{-1}) and Period 3 (0.108 ± 0.013 kg DM · 100 kg BW^{-1} · h^{-1}). Periods 2 and 3 were not significantly different ($P = 0.274$) from each another.

Overall mean DMI during the first 4-h period was greater ($P < 0.0001$) than the second 4-h period (0.148 vs. 0.093 ± 0.010 kg DM · 100 kg BW^{-1} · h^{-1}). Mean DMI rate was greater ($P < 0.05$) in the first 4-h period as compared to the second 4-h period during period 1, but was not different during periods 2 and 3.

The chemical composition of the forage and the initial HM DM in cell A and B within each period is shown in Table 2. The mean initial HM DM (kg DM/m^2) in the DMI cells was 0.321, 0.140, and 0.281 ± 0.015 kg DM/m^2 for periods 1, 2, and 3, respectively. Mean initial HM DM was significantly different between periods ($P < 0.001$). The mean initial HM DM for period 1 was greater than that of period 2 (0.321 vs. 0.140 ± 0.014 kg DM/m^2) ($P < 0.001$) and tended to be greater than period 3 (0.321 vs. 0.281 ± 0.013 kg DM/m^2) ($P = 0.053$). Furthermore, period 3 was greater than period 2 (0.281 vs. 0.140 ± 0.013 kg DM/m^2) ($P < 0.001$). However there was no significant difference between cells A and B ($P = 0.864$) within each period ($P = 0.918$).

The mean DM of forage in the DMI cells was 28.22, 45.19, and 27.50 ± 0.700 % for periods 1, 2, and 3 respectively. There were significant differences in the DM of cells between periods ($P < 0.001$). The DM in period 1 was less ($P < 0.001$) than period 2 (28.22 vs. 45.19 ± 1.093 %) and the DM in period 2 was greater ($P < 0.001$) than period 3 (45.19 vs. 27.50 ± 1.093 %). Periods 1 and 3 were not significantly different ($P = 0.722$).

The mean DE concentration of forage in the DMI cells was 2.12, 2.13, and 2.25 ± 0.013 Mcals/kg DM for periods 1, 2, and 3 respectively. The DE concentration (Mcals/kg DM) in period 1 was significantly less than in period 3 (2.12 vs. 2.25 ± 0.013 Mcals/kg DM). The DE concentration in period 2 was also significantly less than that in period 3 (2.13 vs. 2.25 ± 0.013 Mcals/kg DM). All other fractions are summarized in Table 2.

Experiment 2

Mean BW of all horses increased from d-0 to 42 by 7.9 ± 17.91 kg (time; $P = 0.013$) in period 1, decreased from d-0 to 35 by 11.5 ± 16.98 kg (time; $P < 0.001$) in period 2, and decreased from d-0 to 42 by 44.44 ± 17.45 kg (time; $P < 0.001$) in period 3. Mean BW was not affected by treatment or treatment x time interaction in any of the three periods. However, when analyzed within a treatment, the BW of RES horses decreased from d-0 to 42 by 6.5 ± 3.8 kg in period 1 but was not affected by time (time; $P = 0.114$). In period 2, the BW of RES horses decreased from d-0 to 35 by 15.0 ± 3.46 kg and was affected by time (time; $P < 0.001$). Similarly, in period 3, the BW of RES horses decreased from d-0 to 42 by 39.5 ± 6.39kg and was affected by time (time; $P < 0.001$).

Mean initial HM DM of pastures grazed in EXPT2 are shown in Tables 5, 6 and 7 and Figure 7 and were not different between treatments in all three periods. However, mean intitial herbage mass was greater (pasture; $P = 0.017$) in pasture 2 compared to pasture 1 during period 3.

Mean HM DM differences are shown in Figure 7. Mean HM DM difference (HM difference between pre- and post-grazing across both pastures) was influenced by treatment (Treatment; $P = 0.018$) (RES vs UNRES: 0.213 vs. 0.274 ± 0.013 kg DM/m^2, respectively), but not by pasture or treatment x pasture interaction ($P = 0.101$, 0.715, respectively). Mean HM DM difference was not affected by treatment, pasture, or treatment x pasture interaction during periods 2 and 3 , ($P = 0.597$, 0.410, 0.509, respectively).

Mean chemical composition of forage in all pastures from each period is summarized in tables 4, 5, and 6 for periods 1, 2, and 3, respectively.

During period 1, mean DM of pasture 1 was less ($P < 0.001$) than pasture 2 (32.7 vs. 39.8 ± 0.61 %, respectively). There was also a significant effect of treatment ($P = 0.046$) with UNRES having greater DM than RES (37.2 vs. 35.2 ± 0.568 %, respectively). However, there was no significant effect of treatment by pasture interaction ($P = 0.624$). During period 2, the mean DM of pasture 1 was greater ($P < 0.001$) than pasture 2 (46.72 vs. 37.02 ± 1.138 %, respectively) as well as pasture 3 ($P < 0.001$) (46.72, vs. 33.36 ± 1.138 %, respectively). Furthermore, pasture 2 was greater ($P = 0.030$) than pasture 3 (37.02 vs. 33.36 ± 1.138 %, respectively). There was no significant effect of treatment or treatment x pasture interaction ($P = 0.824, 0.504$) respectively. During period 3, mean DM was not affected by pasture, treatment, or treatment by pasture interaction ($P = 0.117, 0.483, 0.490$, respectively).

During period 1, mean DE concentration of pasture 1 was less ($P < 0.019$) than pasture 2 (2.185 vs. 2.25 ± 0.017 Mcals/kg DM, respectively). There was no significant effect of treatment or treatment x pasture interaction ($P = 0.400, 0.169$, respectively). During period 2, mean DE concentration of pasture 1 was greater ($P < 0.002$) than pasture 2 (2.17 vs. 2.02 ± 0.036 Mcals/kg DM, respectively) as well as pasture 3 ($P = 0.025$) (2.17 vs. 2.07 ± 0.036 Mcals/kg DM, respectively). There was no significant effect of treatment or treatment x pasture interaction ($P = 0.222, 0.060$) respectively. During period 3, mean DE concentration

of pasture 1 was greater ($P = 0.018$) than pasture 2 (2.07 vs. 2.00 ± 0.019 Mcals/kg DM, respectively). There was no significant effect of treatment or treatment x pasture interaction ($P = 0.453$, 0.409, respectively).

During period 1, mean acid detergent fiber (ADF) was not affected by treatment, pasture, or treatment or pasture interaction ($P = 0.136$, 0.281, 0.890, respectively). During period 2, mean ADF was not affected by treatment, pasture, or treatment or pasture interaction ($P = 0.679$, 0.680, 0.442, respectively). During period 3, mean ADF of pasture 1 was less ($P = 0.025$) than pasture 2 (40.71 vs. 42.85 ± 0.588 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.924$, 0.228, respectively).

During period 1, mean neutral detergent fiber (NDF) of UNRES was greater ($P = 0.047$) than RES (58.35 vs. 57.15 ± 0.342 %, respectively). There was no significant effect of pasture or treatment by pasture interaction ($P = 0.802$, 0.977, respectively). In period 2, mean NDF was not affected by treatment, pasture, or treatment by pasture interaction ($P = 0.786$, 0.455, 0.580, respectively). During period 3, mean NDF of pasture 1 was less ($P = 0.007$) than pasture 2 (64.74 vs. 67.29 ± 0.648 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.906$, 0.366, respectively).

During period 1, mean non-fibrous carbohydrate (NFC) of pasture 1 was less ($P < 0.001$) than pasture 2 (16.08 vs. 20.06 ± 0.493 %, respectively). During period 1, there was also a significant effect of treatment x pasture interaction ($P = 0.046$). The rate of increase in

forage NFC content from pasture 1 to 2 was greater for RES ($P < 0.001$) than for UNRES (4.54 vs. 3.42 ± 0.315% change, respectively). However, during period 1, there was no effect of treatment ($P = 0.327$) on mean NFC. During period 2, mean NFC of pasture 1 was greater ($P < 0.001$) than pasture 2 (16.22 vs. 10.41 ± 0.837 %, respectively) as well as pasture 3 ($P < 0.001$) (16.22 vs. 11.14 ± 0.837 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.658, 0.457$, respectively). During period 3, mean NFC was not affected by pasture, treatment, or treatment by pasture interaction ($P = 0.134, 0.197, 0.661$, respectively).

During period 1, mean crude protein (CP) of pasture 1 was greater ($P = 0.001$) than pasture 2 (15.37 vs. 13.24 ± 0.353, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.895, 0.381$, respectively). During period 2, mean CP of pasture 1 was less ($P = 0.003$) than pasture 2 (14.76 vs. 18.58 ± 0.766 %, respectively) as well as pasture 3 ($P = 0.003$) (14.76 vs. 18.58 ± 0.766 %, respectively). There was no effect of treatment or treatment by pasture interaction ($P = 0.836, 0.586$, respectively). During period 3, mean CP was not affected by pasture, treatment, or treatment by pasture interaction ($P = 0.143, 0.364, 0.295$, respectively).

During period 1, mean crude fat of pasture 1 was greater ($P = 0.002$) than pasture 2 (2.44 vs. 2.13 ± 0.035 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.235, 0.885$, respectively). During period 2, mean crude fat of

pasture 1 was less ($P < 0.001$) than pasture 2 (2.18 vs. 2.60 ± 0.072 %, respectively). Furthermore, mean crude fat of pasture 2 was greater ($P < 0.001$) than pasture 3 (2.60 vs. 2.24 ± 0.072, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.757$, 0.626, respectively). During period 3, mean crude fat of pasture 1 was greater ($P = 0.001$) than pasture 2 (3.31 vs. 2.69 ± 0.086 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.815$, 0.071, respectively).

During period 1, mean ash percentage of pasture 1 was greater ($P < 0.001$) than pasture 2 (8.48 vs. 6.83 ± 0.247 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.630$, 0.429, respectively). During period 2, mean ash of pasture 1 was less ($P < 0.001$) than pasture 2 (8.81 vs. 11.82 ± 0.552 %, respectively). Furthermore, mean ash of pasture 2 was greater ($P = 0.002$) than pasture 3 (11.82 vs. 9.55 ± 0.552 %, respectively). There was no significant effect of treatment or treatment by pasture interaction ($P = 0.934$, 0.667, respectively). During period 3, mean ash was not affected by pasture, treatment, or pasture by treatment interaction ($P = 0.223$, 0.457, 0.460, respectively).

Discussion

The NRC (2007) estimates that pasture VDMI ranges from 1.5 to 3.1 % BW/day. Assuming a mature weight of 500 kg and 15 hr of grazing time each day, this estimate corresponds to a DMI rate of 0.10 to 0.21 kg DM · 100 kg BW^{-1} · h^{-1}. The mean hourly DMI rate over all periods estimated in the current study (mean ± std. dev.; 0.121 ± 0.04 kg DM · 100 kg BW^{-1} ·

h^{-1}) is slightly less than an estimate for yearlings grazing orchardgrass pasture when scaled to BW (0.164 kg DM · 100 kg BW^{-1} · h^{-1}) (Duren et al., 1989). Although the same method of estimation was used in both the current study and that of (Duren et al., 1989) direct comparisons between the two studies are not likely the most accurate, because of the difference in physiological state of the horses (current study – maintenance; Duren et al., 1989)– growing). The current estimate is lower than that observed by (Duren et al., 1987) for yearlings grazing fescue (0.31 kg DM · 100 kg BW^{-1} · h^{-1}) or (1.06 kg DM/h), and by (Cantillon, 1986) for mature horses grazing tall fescue (0.30 kg DM · 100 kg BW^{-1} · h^{-1}) or (1.49 kg DM/h) or alfalfa (0.33 kg DM · 100 kg BW^{-1} · h^{-1}) or (1.64 kg DM/h) (methods of estimation not available). Most other estimates present in the literature do not report hourly intake on a BW basis. Thus, calculations were made from the estimates within the literature in order to express the hourly intake on a BW basis. When horse BW was not available, a BW of 500kg for mature horses was used estimation. The current mean estimate of hourly DMI over all periods falls within the range of estimates reported by Moffitt et al. (1987) of (0.11, 0.17, and .16 kg DM · 100 kg BW^{-1} · h^{-1}) across the months of December, May, and August, respectively, for 2-year-old horses grazing fescue or orchardgrass. The current estimate is greater than the estimates of Ince et al. (2005) using welsh ponies (0.087 kg DM · 100 kg BW^{-1} · h^{-1}). However, the ponies were fed supplemental hay, which may have resulted in lower intakes as compared to the present study. The current estimate is similar to that of (Grace et al., 2002a) for yearlings grazing ryegrass/white clover pasture in mid to late spring (0.13 kg DM · 100 kg BW· h^{-1}) and weanlings grazing perennial ryegrass/white clover in the fall (0.122 kg DM · 100 kg BW· h^{-1}). However, it is less than the estimate of (Grace et

al., 2002b), (0.162 kg DM · 100 kg BW· h^{-1}) for lactating Thoroughbred mares grazing ryegrass/white clover pasture in late spring.

It is likely that the differences in DMI rate between the current values and those in the literature are due to differences in physiological state of the horses as well as differences in forage characteristics such as plant species and chemical composition, especially in the case of horses grazing alfalfa (Cantillon, 1986).

Muzzling the horses 12 h prior to the start of the current experiment may have caused an increase in the rate of DMI over the 8-h grazing period that would not normally be observed. Horses in other experiments were managed in different ways, some were fed supplemental hay and were housed in stalls except for the few hours they grazed (Ince et al., 2005), some were exercised (Duren et al., 1989), some received supplemental hay and grain (Duren et al., 1989), and some were simply put on pasture for an entire 24-h period. As such, it is hard to directly determine how the management technique in the current study and those of other studies compare in terms of affecting DMI.

The differences in DMI rate between the first 4-h period and the second 4-h period observed in period 1 are likely due, in part, to the fact that the horses had been fasted for 12 h prior to the first 4-hr period. While not significant, DMI rate was numerically greater during the first 4-h period than the second 4-h period in both period 2 and 3. While the 12-h fast likely eliminated any residual effects from differences in DMI immediately prior to the experiment,

it may have also caused an increased DMI rate, especially during the first 4-hr period. However, using the mean hourly DMI rate from the entire 8-h to calculate required grazing time for the RES treatment during each period of EXPT2 should have helped eliminate any artificial elevation in the calculated required grazing time as a result of the increased hourly DMI rate from the first 4-hr period.

The hourly DMI rate over an 8-h period was greater in period 1 than 2 and 3. One potential reason for this difference was the difference in available herbage mass between the three periods. The differences in DMI and available herbage mass between the three periods follow the same trend, with the greatest DMI and available herbage mass in period 1, the least in period 2, and the median in period 3. Because of the decrease in available forage during period 2, the mass per bite would have decreased and would lead to a decrease in consumption efficiency. Furthermore, there was also an increase in experimental error during period 2, as it was more difficult to detect differences with such small amounts of forage mass and decreased height. Another potential cause of this difference in rate between periods was the difference in forage characteristics between periods. The forage within the DMI cells was very different between periods 1, 2, and 3. In period 1, the forage was dense, leafy, lush, dark green, and had a height of ~ 65 cm. In the winter, the forage was much shorter (~20 cm) and was a mixture brown and green grass. During period 3, the forage had a height of ~ 85 cm and was mature (seed heads present). There was some regrowth at the base, but it was far below the tops of the more mature forage. In period 3, it is possible that because of the maturity of the forage, there would have been experimental error associated

with trampling. Along with this, another potential factor, although relatively small could have been forage DM. The period where DM was the highest was also the period where DMI rate was the lowest, which reflects the dormant state of the forage. While this could have had an effect, and some authors have shown that increased DM is consequent with lower intakes, it is more likely that the available herbage mass was the most influential factor in affecting DMI rate. Arnold and Grassia (1982) stated that horses generally prefer (young) green herbage instead of brown (old) herbage. In each of the periods of this study, the forage was in a different stage of growth or maturity, as shown in Table 2. While nearly all chemical analysis components differed between at least two periods, DE does not appear to have affected DMI rate as there was no significant difference btw the DE in period 1 and period 2.

Another potential reason that period 1 was greater than 2 and 3 was the relatively fast rate of intake during the first 4-h in period 1. The DMI rate during the first 4-h of period 1 was significantly different than all of the other rates from the first 4-h grazing time. A possible explanation for this involves several factors. First, the horses were likely hungrier from having been muzzled for 12 h prior to grazing cell A. The effect of period x cell was caused by the significant difference in intake of cell A and B in period 1 as compared to period 2 or 3. This was likely an effect of the optimal grazing conditions in experiment 1. Overall, the effect of cell, where DMI in cell A is greater than cell B is mostly a result of the difference in period 1 between cell A and B, which was not the case in the other periods.

Furthermore, the forage in cell A and B of period 1 had never before been grazed. This particular stand of fescue had been planted one-year prior, and had never been grazed by horses. It had been harvested for hay approximately 2 months prior to the experiment and had been fertilized approximately 1.5 months prior to the experiment. As such, the forage was not only extremely dense, as evidenced by the greatest initial HM DM (Table 2), but also relatively immature, without the presence of seed heads. Therefore, there was likely a higher leaf : stem ratio in period 1 than the subsequent periods, which could have affected DMI rate. This is supported by the findings of McMeniman (2003) who suggested a relationship between the percentage of leaf in the diet and the dry matter intake as well as Fleurance et al. (2001) who suggested that horses select herbage on the basis of stage of growth rather than botanical species. Furthermore, in a study investigating the influence of sward height on diet selection, Naujeck et al. (2005) found that horses resided significantly longer and took more bites of forage on patches of pasture with long grass (15 cm) than those with short grass. Height of forage appeared to be greater in period 1 and 3, as evidenced by the greater initial HM DM value in these two periods. Thus, the taller grass in these periods (1 and 3) could have affected the DMI rate, as has been shown by a number of other investigators (Fleurance et al., 2001; Hansen et al., 1987a; Naujeck et al., 2005). Naujeck et al. (2005) found that the quality of young re-grown grass had an influence on the diet selection of horses, but that the horses likely preferred forage that had not been cut, and therefore provided a more "natural" sensation (i.e. tactile, olfactory) while grazing.

Horses also aim to avoid herbage contaminated with soil Naujeck et al. (2005) or patches where feces had been deposited (Fleurance et al., 2001). Each of these factors can be applied to the grazing cells in period 1, as they had never before been grazed (and hence were not contaminated with urine or feces), and were at stage of growth where the forage was "natural" in appearance (i.e. few residual effects from the making of hay).

Furthermore, it is also possible that the DMI rate was affected by the season of the year. A number of studies have found that time spent grazing varies from season to season, and in one study in particular, horses spent nearly 10% more of their time grazing in September (fall) than they did in December (winter) (Hansen et al., 1987b). Since period 1 of EXPT1 began in October, it is possible that the horses were adapted to grazing for more of the day in comparison to February or May, and hence were hungrier after having been muzzled for 12h. In addition, Berger et al. (1999) found that horses graze in a diurnal pattern and that seasonal changes in ambient temperature and day length altered these rhythms. Each of these variables would have differed between seasons and could have contributed to the difference in DMI rate.

There was a significant effect of period in all of the proximate analysis components with the exception of NDF. As such, it is likely that hourly DMI rate varied between periods in part because of the differences in forage characteristics. There are also likely interactions between the chemical components of forage that are beyond the scope of these analyses. However, it appears likely that forage DM may influence DMI rate, as the lowest DMI rate

corresponded to the period during which forage DM was the highest (period 2). This observation is supported by the finding of Fleurance et al. (2001) that forage is selected on the basis of structure (growth stage) rather than botanical species. Younger, more immature forages likely have higher moisture content than more mature forages. Therefore, the fact that the period when DM was the highest in the forage was also the period when DMI rate was the slowest suggests that this theory may be valid.

Interestingly, the lowest concentrations of NFC, WSC, and ESC in the forage corresponded with the greatest DMI rate. This seems counterintuitive, as these fractions include components, such as of mono- and disaccharides, oligosaccharides (including fructan), and starch (NRC, 2007), that are typically thought to make forages more palatable to horses. However, there is possibility that collection procedures during period 1 could have introduced error into this fraction. During period 1, samples were not frozen as quickly as they were in periods 2 and 3 and potentially were exposed to sunlight for a few hours. McCown et al. (2009) found that tall fescue samples that were left in the sun prior freezing and analysis for WSC and ESC had lower WSC and ESC values than those that were frozen or kept in a cooler on ice McCown et al. (2009).

While there were typically small numerical differences in most of the proximate analysis components between cell A and B within a period, most of the differences were not statistically significant. While CP and ash both had a significant effect of period x cell interaction, the numerical differences were still quite small and did not likely contribute to

any difference in DMI between cells. This is supported by the findings of Dulphy et al. (1997) who found that VDMI in horses trough-fed forage was not influenced by CP. In period 1, where there was a significant difference between the DMI in the first and second 4-h periods, there was a tendency for an effect of period x cell interaction on DM, indicated by an increase in DM from cell A (27.12% DM) to cell B (29.31%). It is possible that this small numerical change in DM % from cell A to B affected DMI rate, but typically, with increased moisture content, horses will decrease their DMI rate, due to the increased feeling of satiety from the moisture in the forage. This effect was suggested to play a role in a study conducted by Ince et al. (2005), where forages with very high moisture contents were fed to ponies. Ince et al. (2005) suggested that high moisture content of forages precludes higher DMI due to gut fill. Regardless, it is unlikely that such a small numerical change in DM would have a large impact on DMI rate.

While there were numerical differences in several of the pastures that suggested that the HM DM difference was greater for UNRES than RES, there was only a significant difference in period 1, where the mean HM DM difference was less for RES than UNRES. Furthermore, there were also pastures where the numerical differences suggested that the HM DM decreased at a faster rate for RES than for UNRES. While not significant, these results seem to indicate that the sampling technique used to determine HM DM in the EXPT2 enclosures was unable to detect small differences in the HM DM of each enclosure. Moreover, the results suggest that when HM DM is at a relatively low level, the method used here is not able to detect differences, and may even misrepresent the HM DM present, depending on the

random sampling areas. During EXPT2, it was observed that "lawns" and "roughs" appeared in many of the individual enclosures. As mentioned previously, Fleurance et al. (2001) and Naujeck et al. (2005) both speculate that the appearance of these "roughs" and "lawns" may be the result of horses wanting to avoid areas where feces were deposited, or moreover that they simply prefer to graze the lawns where more immature, and likely higher quality (lower fiber, higher CP) forage is present (Fleurance et al., 2001). Because of the presence of these "roughs" and "lawns", especially in period 3, it is possible that in order to accurately and precisely represent the HM DM in each enclosure, a greater number of random samples should have been taken from each enclosure. Because of the large size of the enclosures used in EXPT2, there is a chance that the four randomly chosen $1m^2$ subplots were not able to capture the small difference in residual HM DM between the RES and UNRES group.

In period 2, where initial HM DM was numerically less than other periods, it is possible that the HM DM remaining after the horses had grazed each pasture was inadequate to indicate differences between treatments. Another possibility is that RES horses were able to increase their DMI rate during their grazing time such that they could consume nearly the same amount as the UNRES horses over the period of 24 h. While this is not supported by the numerical differences in HM DM in most of the pastures, there are a few pastures where the numerical differences, while not significant, indicate that RES consumed a greater amount than UNRES. It is possible that by muzzling the horses for an extended period of the time during the day, the horses tried to overcome their decrease in grazing time by increasing their DMI rate or bite mass. Duren et al. (1989) observed that yearlings grazing orchardgrass were

able to modify bite rate (bites/min) as well as the bite mass (mg/bite), such that some horses took fewer but larger bites while some took more bites of smaller mass. This modification in grazing behavior allowed all horses to have similar intakes over time, but with changes in bite rate and mass (Duren et al., 1989). This could have also been the case with the RES horses in this experiment, which may have increased their DMI rate to one greater than the UNRES. Nonetheless, these results suggest there typically was some conservation of forage by restricting grazing and that it is possible that RES horses were able to modify their normal DMI rate.

There was a significant effect of time on the mean BW of all horses in all periods. The increase in the mean BW of all horses over time in period 1 was relatively small (i.e. ~8 kg in 42 d), whereas the decrease in period 2 (~12 kg in 35 d) and period 3 (~44 kg in 42d) where relatively greater. In period 1, when the BW of RES are compared separately, BW of RES in period 1 did not have a significant change. The lack of significant change in BW among the RES horses suggests the hourly DMI obtained in period 1 is valid. It was hypothesized that UNRES would eat more grass and gain significantly more weight than RES and would act as a control. However, there was no significant effect of treatment. While UNRES did not gain more weight than RES, they did consume more forage, which is supported by the greater HM difference in UNRES versus RES, with similar forage DE concentrations. The increased consumption of forage by the UNRES horses was not a function of having more available herbage mass or a greater DE. In period 2, all horses lost weight. The drop in BW suggests that the estimate obtained during period 2 may not have been valid or that other factors may

have contributed to the decrease in BW. The decline in BW suggests that the horses were in negative energy balance. Therefore, we either did not allow the horses to consume adequate amounts of forage or the horses had a higher requirement than what we estimated. One factor that could have affected the change in BW was the relatively low quantity of available herbage during period 2. One possible reason for a lack of treatment effect is the synchrony of grazing, meaning that the behavior of the RES horses may have affected the behavior of the UNRES horses. It was observed, but not quantified, that horses in both treatments congregated at the water tanks and along the fence line. Furthermore, RES horses were permitted to graze for 17 hr/day which, according to a number of estimates, is toward the upper limit of the time horses would spend grazing normally. Therefore, both groups likely grazed for similar amounts of time. It is also possible that the change in available herbage mass could have caused a decrease in BW as the horses had less herbage to graze. There were not any differences across pastures or between treatments, but the available herbage mass was much less than in periods 1 and 3. While the DE of the pastures did decrease over time in period 2, this should not have caused the decrease in BW as the grazing time was adjusted to account for theses decreases. In period 3, RES horses lost weight over time, indicating that the associated DMI rate estimate was not valid. However, available herbage mass and DE content did not appear to have an effect on this decrease in BW. There was no significant effect of DE to account for the decrease in BW. Furthermore the loss of BW was not a function of available herbage mass, as the amount of herbage mass had a tendency to increase over time. In the case of the pastures used in period 3, more available forage was not necessarily better for maintenance of BW and the available forage was similar to period

1. The forage present in these pastures was very mature with seed heads and a very fibrous texture. Therefore, the decrease in BW was likely an effect of the forage characteristics rather than lack of available forage (which was likely the main cause of decreasing BW in period 2). In period 3, both UNRES and RES horses lost weight over time. However, there were no treatment differences. One potential reason for the lack of a treatment effect was that similar to period 2, horses were grazing for about 14 hrs which is near the upper time limit observed in free ranging horses. Thus it is possible that even though the RES group was muzzled, they were still grazing for a similar amount of time as UNRES. Initial HM values indicated that there were no differences in the availability of forage to either treatment and that horses were not limited by herbage mass, but rather a different factor. There was no difference in the initial and final yield between the treatments. This likely indicates that the horses in both groups were grazing the enclosures similarly. The wide variability in HM consumed is likely due to the way the horse grazed their enclosures in this period. Lawns and roughs were observed to have appeared in the pastures during this period. It is likely that the horses tended to spend most of their time on the lawns rather than the roughs. Therefore, the method of HM estimation may not have been able to capture small differences in period 3 because of the small number of samples obtained from such a large enclosure. It is also interesting to note that the NDF values were greater in period 3 than in period 1 or 2. This increase in maturity of the forage may have decreased the palatability.

Lewis suggests that common causes of BW loss include: 1) Poor quality or inadequate amounts of forage available, 2) Dental problems, 3) excessive amounts of internal or external parasites, 4) high energy needs due to lactation or hard work, 5) prolonged hot/humid weather which increases energy needs and decreases feed intake, 6) chronic disease that decreases intake or utilization of feed, or 7) the horse's being low in the pecking or dominance order in a group and, as a result being chased away or, because of fear, staying away from adequate feed (Lewis, 1995). All horses used for this trial were considered to be healthy and at a physiological state of maintenance. Therefore, it is unlikely that high energy needs due to differences in physiological state or chronic disease impacted BW.

Since horses were housed in individual enclosures, DMI should not have been affected by dominance order. However, it is possible that changes in BW were affected by the other factors suggested by (Lewis, 1995). This is especially the case with the dentition of the older horses in the group. Dental abnormalities are common in older horses (Graham, 2002; Paradis, 2002) and can limit the ability to prehend and chew feed, decrease the digestibility of nutrients, and lead to substantial loss of BW. In the discussion that follows, these, and other factors that could have affected changes in BW are investigated at length.

One possible reason for the changes in BW during each period is the normal fluctuation of horse BW throughout the year. Berger et al. (1999) observed a group of semi-feral Przewalski horses and found that horse BW fluctuated in a pattern similar to the changes in air temperature. Over the period of one year, Berger et al. (1999) determined that BW of the

Przewalski horses fluctuated by ± 30 kg. Berger et al. (1999) observed the following trends:

Spring: horse BW is at its lowest level; mean daily feeding time is higher than any other season

Summer: horse BW is increasing; feeding time drops to the minimum

Autumn: horse BW is at its maximum: feeding level is generally greater

Winter: horse BW is decreasing; feeding level is generally high

In Berger's study, BW reached its maximum in September (highest temperatures were recorded in July) and BW was lowest in March (lowest temperatures recorded in January). These results suggest a time lag in changes of BW corresponding to changes in air temperature. The results of the current study followed this trend to some extent, with greatest mean BW in the fall (period 1-October) after the high temperatures of July and August (weather.com), lowest mean BW in the winter/spring (period 2-February) after the low temperatures of December and January (weather.com), and mean BW at an intermediate level in the spring/summer (period 3-May). However, the change in mean BW over the duration of period 3 did not necessarily follow this pattern, as it decreased over the 42 d (from spring into summer). The mean BW changes in the other periods did seem to follow this trend. As such, the mean changes in BW may have been more highly correlated to seasonal and environmental effects than to the estimate of DMI rate.

Separately, Havel (2001) suggested that in adult animals and humans, BW tends to remain within a relatively narrow range, despite large day-to-day fluctuations. While all horses were muzzled for 12 h prior to being weighed each week in periods 2 and 3, it is possible that

residual effects from differences in daily DMI (day-to-day fluctuations) on the day prior to weighing could have affected horse BW. This is especially true for period 1, where only the RES horses were muzzled prior to being weighed each week. However, while not muzzling the UNRES horses prior to weighing would affect the accuracy of the measure, it likely would not have affected the ability to detect a change over time assuming the error in measurement was consistent each time the horses were weighed. Determination of actual body mass, excluding the effect of gut fill, is difficult to measure as feed must be withheld for longer periods of time which may have detrimental effects on gastrointestinal health. While the current method aimed to reduce these residual effects, it is possible that they were not entirely eliminated and could have affected the BW measurements.

Another potential reason for the changes in mean BW across periods is the inability to change calculated grazing time at the same rate that the BW and DE were changing. Stated more simply, because of the time required to analyze samples for DE content, there was a time lag in changing required grazing time to correspond with changes in pasture DE concentration. Furthermore, the calculated grazing time for RES was always based on the initial (day 0) BW measurement. Therefore, weekly changes in BW were not taken into account when determining required grazing time for RES. Because of this, time required to maintain the updated BW would have changed slightly from week to week in accordance with changes of BW and pasture DE. Our inability to update required daily grazing time in "real time" with changes in horse BW and pasture DE may have affected the changes in mean BW that were observed in each period. However, this is not likely a factor since the

horses were grazing for a period of time that was similar to the maximum grazing time of feral horses. In addition, in order to maintain compliance with farm staff, it was not logistically feasible to change the required grazing time each week. Error may also have been introduced to this measurement because of the need to "round" the required grazing time up or down to the nearest hour to maintain compliance with farm staff.

It is also possible that changes in grazing behavior occurred as a result of the experimental design and that changes in behavior had an effect on daily DMI, and hence, horse BW. During EXPT1, it was observed during all periods that some horses grazed more normally than others in the 5 m^2 cells. It was not typically the same horse across each period, so no horses were excluded from the analyses on the basis of their behavior. However, it was observed that some horses had increased vocalization and movement during the 8-h period. It is possible that the relatively small size of the 5 m^2 cells prevented horses from their typical grazing behavior of tearing a mouthful of grass, moving a few steps while chewing, and then stopping to take another bite (Houpt, 1990). The square shape and relatively small size of the 5 m^2 cells could have precluded the horses' ability to move around normally while grazing.

Furthermore, Arnold and Grassia (1982) and Houpt (1990) both suggest that horses are stimulated to eat by visual contact with other horses and prefer to graze while in the presence of other horses. Arnold and Grassia (1982) also observed that horses are typically close to at least one other horse while grazing and resting. Similarly, it has been observed that when

horses are isolated from direct visual contact with other horses, they spend only half as much time grazing as horses with visual contact of other horses (NRC, 2007). While all horses in this study had visual contact of at least one other horse in EXPT1 and EXPT2, it was observed that horses often congregated at the fence line or water tanks, and in general, preferred to be in close proximity to other horses. It is possible that horses spent less time grazing in EXPT1 and EXPT2 than they would have if turned out as a herd. The effect of visual contact appeared to have a greater effect on certain horses than on others, as evidenced by pacing and vocalization in specific horses, and could have dramatically influenced the effect of treatment and treatment x time in RES or UNRES as a result of small numbers of animals.

It is also possible that typical grazing behavior was interrupted due to the time of day the RES horses were permitted to graze in both EXPT1 and EXPT2. In EXPT1, the horses were permitted to graze from about 8 a.m. to 4 p.m. because of the relative ease of conducting the trial during daylight hours. During EXPT2, calculated grazing time was very different between the three periods. Because of the difference in length of grazing time, it was not possible to have the horses grazing during the same time-of-day in all three periods. For example, in period 1, horses were muzzled overnight, and hence were free to graze during daylight hours; in period 2, horses were muzzled during the middle of the day and hence were free to graze in the afternoon, evening, night, and morning hours; in period 3, horses were muzzled during the day and thus were able to graze in the evening and nighttime hours. It is possible that by muzzling the horses at certain times of day, we altered their typical

grazing behavior and thus, may have affected DMI. This is supported by Berger et al. (1999) who observed a group of semi-feral Przewalski horses, and found that daily grazing time varied with seasonal changes in ambient temperature and day length and that horses graze in a diurnal pattern. Thus, it is possible that the horses had a depressed intake (and thus lost BW) in EXPT2 of periods 2 and 3 because of grazing restriction during a certain time of day. It is also important to note that while the grazing muzzles were useful in restricting grazing, it is impossible to know how much forage horses were able to consume through the 2.54 cm opening at the base of the muzzle. It is likely that the amount consumed while wearing the muzzle was small and varied from horse to horse as well as from period to period. It was observed that when forage sward height was shorter, horses seemed to have an easier time consuming forage while wearing the muzzle as compared to periods where the sward height was much taller.

Another potential reason for the changes in mean BW across periods was the lag time between the start of EXPT1 the end of EXPT2 (the entire duration of each period). From the time the DMI estimates were made to the end of the 42 days of EXPT2 was typically between eight to ten weeks, depending on the period (i.e. period 1 = October 15 to December 12, 2008). The reason for the delay between EXPT1 and EXPT2 in each period was the time required to have forage samples analyzed for DE content, make calculations, and prepare for EXPT2. It was necessary to know the DE concentration in order to calculate required grazing time for RES. It is highly likely that hourly DMI rate changed from the time EXPT1 was conducted to the end of EXPT2 in each period. During this eight to ten week duration, a

number of factors that likely affect hourly DMI rate, would have changed, including environmental conditions, forage characteristics, and day length. These factors are investigated at length in the following discussion.

While beyond the scope of this discussion, it is likely that both short-term signals from the GI tract (i.e. cholecystokinin and GI stretch receptors) as well as adipokines released from adipose tissue and long-term signals such as leptin were involved in the regulation of appetite during each component of this experiment. These short-term and long-term signals act in concert to regulate energy balance, satiety, and meal termination (Havel, 2001).

Lastly, it must be noted that following completion of the trial in July 2009, it was determined that the eight horses involved in this particular study had last been treated for parasites in October of 2008, and were possibly harboring gastrointestinal parasites in periods 2 and 3 of the experiment. If in fact the horses were infected with gastrointestinal parasites during periods 2 and 3, it is likely that this would have played a role in the BW loss during these periods.

In period 1, the small BW change in the RES group was expected and suggests the intake estimate from the present experiment is valid under the experimental conditions. The lack of difference in BW change between treatments in period 1 was not expected, given UNRES had a greater DMI than RES during the second 21-d period, as indicated by a significant effect of treatment x time on HM DM. One likely reason for the lack of difference in BW

change is that three of the four UNRES horses were the older horses of the group. The four UNRES horses ranged in age from 9 to 25 yr of age with an average of 18 ± 7.0 yr (mean ± SD); whereas the RES horses ranged in age from 8 to 22 yr of age with an average of 13 ± 6.4 yr (mean ± SD). Since forage digestibility is likely decreased in older horses (i.e., > 20 yr) due to incomplete mastication, and reduced absorptive function (NRC, 2007), the older horses would have had to consume more forage to obtain the same amount of calories as the younger horses. Another likely reason for the lack of difference in BW change is that two of the four UNRES horses were Thoroughbreds while the others were Quarter horses. Since Thoroughbreds often have nervous temperaments or higher levels of voluntary activity, this could have increased the maintenance DE requirements of these horses. This difference in breed may have confounded the BW results. There is also a possibility that the greater DMI in UNRES horses led to an increased passage rate and lowered DM digestibility (Clarke et al., 1990). Furthermore, the UNRES group may have had greater daily energy expenditure as a result of increased activity associated with grazing (NRC, 2007). Since the horses' grazing was restricted by the use of muzzles, they may have spent more time each day standing idle than moving around the enclosure grazing.

Forage characteristics during period 1 may also have affected changes in BW. During period 1, there was a significant effect of treatment on pasture DM. Numerically, the forage grazed by RES had lower DM at each sampling time (pre- and post-grazing) in each pasture, indicating that the difference in DM was not a result of grazing, but rather was a difference that could have impacted DMI. As mentioned previously, it has been suggested that horses

prefer less mature forage versus more mature forage (Fleurance et al., 2001). Since DM of the forage varies with changes in maturity, it is possible that the RES horses had a greater DMI rate than the UNRES horses as a result of this difference in forage DM. Similarly, there was a significant effect of treatment on pasture NDF. Numerically, the forage grazed by RES had lower NDF at each sampling time (pre- and post-grazing) in each pasture, indicating that the effect of treatment on NDF was not a result of grazing, but rather an effect that could have impacted DMI. Fleurance et al. (2001) found that horses tended to prefer shorter grass "lawns" that were higher in quality, as indicated by lower NDF. As such, it is possible that like DM, the RES horses had a greater DMI rate than the UNRES horses as a result of this difference in forage NDF. However, the current results are not supported by the findings of Dulphy et al. (1997) who determined that VDMI in horses is not affected by NDF. Furthermore, forage NFC increased at a greater rate from pasture 1 to 2 for RES horses than for UNRES. Since the components of the NFC fraction are comprised of compounds (i.e. mono- and disaccharides, fructan etc.) that can be considered to increase palatability (Hoskin and Gee, 2004) and intake (Rogalski, 1984), it is possible that similar to DM and NDF, the RES treatment had a greater DMI rate than UNRES as a result of this difference in rate of change of forage NFC. The CP content of the forage decreased from pasture 1 to 2 at a similar rate for both treatments. These results are in disagreement with the findings of Fleurance et al. (2001) and Hansen et al. (1987a) who both determined that horses preferred forage with higher CP. Since mean horse BW increased in period 2 in the current study, it does not appear that the decrease in CP influenced DMI from pasture 1 to pasture 2.

During period 2, the decrease in mean BW over time (~12 kg over 35 d) was not expected and suggests that the intake estimate from EXPT1-period 2 may have been greater than the actual DMI rate during EXPT2 or that other factors were affecting the DMI of all horses during the 35 d duration of EXPT2. In period 2, horses were blocked by age and breed in order to ensure the balance of each treatment for EXPT2. In doing this, the effect of age or breed on the treatment and treatment x pasture interaction should have been minimized. Among the potential factors causing this decrease in weight, effect of season and forage characteristics may have had an impact.

While Hansen et al. (1987b) observed yearling horses grazing for a shorter period of time in December than in September, Houpt (1990) suggests that cold conditions likely increase grazing time. However, Cymbaluk and Christison (1990) suggest that even with an increase in intake, during cold wet weather, horses may be unable to overcome the effects of the weather and may lose weight. Since period 2 was conducted from February to April, it is possible that the relatively cold, wet weather during this period affected DMI and hence, caused a decrease in BW. While cold weather has been suggested to increase intake (Houpt, 1990), wet and windy conditions result in decreased time spent grazing (Rogalski, 1975). Because of the placement of the experimental pastures, wind likely had an effect on DMI, especially during the relatively cold temperatures and wet conditions of February, March, and April. The results of the current study agree with the report of Cymbaluk and Christison (1990), who found that even when fed at 150% of the recommended energy intake, mares exposed to cold, wet weather still lost weight.

Forage characteristics during period 2 may also have had an effect on DMI rate, and hence BW. The difference in forage DM between pastures 1, 2, and 3 in period 2 may have had an effect on DMI rate. Forage DM decreased in each pasture with the highest level in pasture 1 and lowest level in pasture 3. As such, the decrease in BW is not an expected result. As discussed previously, it has been suggested that horses prefer forages with a lower DM percentage (Fleurance et al., 2001). However, if this were the case, the expected result of decreasing forage DM would have been an increase in mean BW. Another forage characteristic that likely affected BW was forage DE concentration. As indicated in Table 6, forage DE concentration was greatest in pasture 1, and least in pasture 2, with pasture 3 having the intermediate value. Even though BW decreased over time, as indicated in Figure 6, it decreased at a faster rate in pasture 2 than in pasture 1. Therefore, it is possible that the However, because of the few number of days spent in each pasture, it is difficult to say for sure that changes in BW were an effect of the characteristics in any specific pasture.

The CP content of the pastures may have attributed to the change in BW as it was greater in pasture 1 than either of the other pastures. As previously mentioned, both Fleurance et al. (2001) and Hansen et al. (1987a) reported that horses prefer to graze pastures with greater CP contents. Therefore it is possible that the lower CP content of pastures 2 and 3 could have depressed DMI in both treatments. The caveat to this is that in both Fleurance et al. (2001) and Hansen et al. (1987a) studies, forage was not limiting, so horses had the ability to express selection preferences. In the case of the current study however, it is unlikely that HM DM was available in great enough quantities during period 2 to allow for similar

selection preferences. Differences in ash content may also have contributed to loss of BW. Ash levels were highest in pasture 2, which suggests that the forage may have been contaminated with soil or feces. As suggested by Naujeck et al. (2005) horses may aim to avoid forage contaminated with soil. The forage most likely to be contaminated with soil is the "lower horizons of pastures", or more simply, the shorter forage. Therefore, since observed forage height and HM DM were far less in this period, it is likely that DMI was depressed as a result of short sward height and contamination with soil or feces. Since the numerical difference of crude fat was relatively small across pastures, it is unlikely that it had a large impact on DMI.

As discussed earlier, decreased HM DM availability may have been the most limiting factor in terms of DMI and hence, BW gain in period 2. The duration of EXPT2 during period 2 was shortened to 35 d as a result of limiting HM DM in the experimental pastures. Not only did the duration of EXPT2 during period 2 require shortening, but it was also necessary to incorporate the use of a third pasture to reach the 35 d duration. Because of this limitation of HM DM, horses grazed each experimental pasture down to a point where differences were difficult to detect with the current method of intake estimation. This suggests that while measuring residual HM DM may yield reasonable results, the method likely is more accurate and precise when HM DM is at a relatively higher level than it was during period 2. With limited HM DM, horses may not have been physically capable of increasing their DMI to a point where they were gaining weight each week, regardless of the palatability of the forage. As is indicated by the initial HM DM levels in Table 6, the amount of forage initially

available for the horses in period 2 was far less than what was available in both period 1 and 3. Furthermore, not only was the available HM DM less, but the observed forage height was far less. Therefore, horses would have had to take more bites, with less mass per bite, over a greater area of pasture (Naujeck et al., 2005). One additional factor is the effect of areas of defecation. Since the experimental pastures had been grazed a few months earlier, it is possible that areas of defecation and urination had residual effects during this period and the subsequent period, thereby reducing the HM DM that the horses were willing to consume. These factors in addition to the effects of weather discussed above, likely depressed both daily DMI and horse BW gain.

In period 3, the relatively large decrease in mean BW (~44 kg over 42 d) was not expected and suggests that the intake estimate from EXPT1- period 3 may have been greater than the actual DMI rate during EXPT2-period 3. It is also likely that other factors such as environmental conditions and forage characteristics could have affected DMI during EXPT2 of period 3. As in period 2, horses were blocked by age and breed in order to ensure the balance of each treatment.

Period 3 was conducted beginning in the month of May and continued until the month of July. In North Carolina, these months of the year are associated with warm temperatures and humid conditions. These environmental conditions could likely have impacted DMI during EXPT2. Berger et al. (1999) observed that in a group of semi-feral Przewalski horses, feeding accounted for 62% of total activity in the spring, whereas it only accounted for 40%

of total activity in the summer (Berger et al., 1999). Since EXPT1 of period 3 was conducted in the middle of May, it is likely that the horses were not greatly affected by air temperature or humidity during EXPT1 of period 3. However, EXPT2 of period 3 was conducted from mid-May to the beginning of July, during which time, mean air temperature would have increased and humidity would have become more of an issue. A number of investigators have suggested an effect of weather conditions on grazing time, with high temperatures and high humidity typically causing a decrease in feed intake and an increase in water intake as horses attempt to stay cool (Cymbaluk and Christison, 1990). It has also been suggested that relative humidity can increase time spent grazing NRC (2007), but it is unlikely that the pairing of high temperature and high humidity would serve to increase grazing time. Cymbaluk and Christison (1990), found that during hot weather, intake may decrease by 15 to 20%, but that feed type is also important. Fibrous feeds often aggravate heat stress as the heat increment of these feeds is relatively high in comparison to that of grains or fat (Cymbaluk and Christison, 1990).

As is indicated in Table 7, the forage grazed during period 3 was the most fibrous of any period and thus may have had an impact on both DMI and horse BW. Furthermore, the placement of the experimental pastures in this study did not provide horses with any shade or respite from biting insects, and as such may have increased both stress associated from heat as well as stress associated with insects. It has previously been determined that insect annoyance should be considered a potential cause of weight loss when pasture quality is not a contributing cause (NRC, 2007).

Forage characteristics during period 3 likely impacted both DMI rate, and hence, the decrease in horse BW. The decrease in forage DE concentration from pasture 1 to pasture 2 could likely have had an effect not only on DMI, but also on BW. Because of the lag time in analyzing the forage samples for DE concentration, adjustments to calculated grazing time for the RES horses could not occur until after they had already been grazing pasture 2 for a period of about one week. Therefore, during this week, the RES horses would have grazed for a period of time that was shorter than required to meet their daily requirements.

Furthermore, as discussed earlier, it has been suggested that horses increase intake when forages are more highly digestible Moffitt et al. (1987) as a strategy to maximize daily nutrient assimilation (Fleurance et al., 2001). In the case of the current study, DE decreased from 2.25 Mcals/kg in EXPT1 of period 3 to 2.08 and 2.00 Mcals/kg in EXPT2. Therefore, it is possible that the DMI rate estimated in EXPT1 was greater than the DMI rate exhibited by the horses in EXPT2, in part because of the decrease in DE concentration and increase in maturity of the forage. This is contrary to the suggestion of Hoskin and Gee (2004) who suggest that VDMI may be a function of DE intake, whereby horses maintain a constant DEI my altering DMI. Instead, the results of the current study align more closely with the suggestion of Lewis that as forages mature (as was the case in both pasture 1 and 2), their palatability and digestible nutrient content decrease rapidly, resulting in a decreased intake of mature forages (Lewis, 1995).

The maturity of the forage grazed by horses in EXPT2 of period 3 is indicated by the relatively high values of NDF in Table 7. The NDF content of the forage in pasture 2 of period 3 was nearly 10% higher than that grazed in periods 1 and 2. This increase in maturity and fiber content of the forage in period 3 likely depressed the DMI. A number of other investigators have observed decreases in DMI with increasing forage maturity and NDF content (Arnold and Grassia, 1982; Fleurance et al., 2001; Lewis, 1995). This was likely the case in the current experiment. Not only was NDF content greater in EXPT2 of period 3 versus EXPT1 of period 3, but it was also numerically greater (statistical significance not determined) than in any of the other pastures across periods. This may be one reason the horses continually lost weight (Figure 6) during each week of EXPT2, because each week the forage was becoming increasingly mature. While Dulphy et al. (1997) found that VDMI in horses that were trough fed forage was not affected by NDF content, it is likely that in the current study there was an affect of NDF on DMI. The data from the current experiment is supported by Frape (2004) who notes that if digesta derived from very coarse, poorly digested, long-fibrous feeds is present in large amounts it will be retained longer in the large intestine and will depress daily DMI. This is most likely the case in the current experiment. While there was a significant decrease in the crude fat content of the forage from pasture 1 to pasture 2, the change was rather small numerically, and likely did not have much affect on DMI.

While not necessarily indicated by the chemical analysis values, the forage in pasture 1 and especially pasture 2 was a mix of very tall (~ 85 cm) mature forage (seed head formed) as well as relatively short immature forage (~ 35 cm). As such, it is possible that sward height impacted DMI in period 3. Fleurance et al. (2001) found that mares spent about 70% of their time grazing short "lawns" that only represented 10% of the total area and avoided patches of tall grass. This finding is also supported by Hansen et al. (1987a), who observed yearlings that spent the majority of their time grazing the immature "lawns" which only represented about 38% of total grazing area and avoiding the areas with tall grass (i.e. "roughs"). While Naujeck et al. (2005) found that horses preferred to graze taller swards, the sward heights used in their experiment were relatively short at 15 cm maximum. In the current study, it was observed that horses first consumed the seed head portion of the mature forage and left the fibrous stem portion standing. After they consumed the seed head, they then attempted to consume the shorter less mature forage at the base of the remaining fibrous stems of the mature forage. It was observed on several occasions that some horses were experiencing eye irritation, presumably caused, in part, by poking of the fibrous stems of the mature forage. As the horses lowered their heads to consume the shorter forage, they had to put their heads below the tops of the tall fibrous stems of the mature forage, which likely caused discomfort. It is also possible that the eye irritation was caused in part by the flying insects.

The results of this study indicate that HM reduction can be used as a method to estimate hourly DMI rate and that the hourly DMI rate estimate from EXPT1-period 1 is reasonable for mature horses grazing tall fescue under the conditions of this experiment. These results

are supported by the lack of significant change in BW of the RES horses in EXPT2. The significant decrease in mean BW of all horses in periods 2 and 3 indicate that the hourly DMI estimates obtained during each corresponding EXPT1 may have been underestimated. However, in period 2, it is likely that in EXPT1 there was relatively high experimental error in estimating DMI because of the small amount of forage in the experimental cells. It is also possible that in period 2 EXPT2, the horses didn't have adequate forage to maintain their BW. In period 3, it is possible that there was decreased palatability leading to a decrease in intake and thus, a decrease in horse BW over time. Another possibility is that the hourly DMI rates obtained in EXPT1 were reasonable, but that environmental factors and forage characteristics in EXPT2 confounded the change in mean BW. Needless to say, these hourly estimates may not be appropriate for horses grazing pasture other than tall fescue, growing horses, or horses in a physiologic state other than maintenance.

The changes in mean BW of all horses in each period indicate that hourly and daily DMI likely vary from day to day as well as with changes in environmental variables, season, and forage characteristics. By withholding feed prior to EXPT1, atypical hourly DMI rates could have been observed. In future studies, it may be useful to adapt horses to grazing for a set time each day prior to the experiment, to replicate a more typical management strategy. While this data suggests that herbage mass reduction is a useful tool for determining DMI, this method may be most accurate when forage HM (kg/m2) is at relatively high density, as was the case in periods 1 and 3. Studies comparing the estimates of hourly DMI rate obtained through different methods such as herbage mass reduction, markers (i.e. n-alkanes),

short term weight changes (after accounting for excretory and insensible losses), and measurement of fecal outputs and known DM digestibilities would be useful in determining the most useful and accurate method to obtain estimates in future studies.

Table 1. Characteristics of experimental horses.

Horse	Breed	Age	Sex
Horse 1 (ADT)	Thoroughbred	22	Mare
Horse 2 (Avalon)	Quarter Horse	9	Mare
Horse 3 (Dee)	Quarter Horse	13	Mare
Horse 4 (Lucy)	Quarter Horse	9	Mare
Horse 5 (Maile)	Thoroughbred	17	Mare
Horse 6 (Sinful)	Quarter Horse	25	Mare
Horse 7 (Suzie)	Quarter Horse	22	Mare
Horse 8 (Tex)	Quarter Horse	8	Gelding

Table 2. Effect of period, cell, and period x cell interaction on tall fescue chemical composition in EXPT1.[a]

	Period 1		Period 2		Period 3			*P*-value		
Item	Cell A	Cell B	Cell A	Cell B	Cell A	Cell B	SE	Period	Cell	Period x Cell
DM	27.15	29.40[b]	44.56	45.81	27.97	27.03	0.859	< 0.001[c,e]	0.129	0.100
DE	2.10	2.14	2.13	2.13	2.25	2.25	0.018	< 0.001[d,e]	0.418	0.399
ADF	34.57	34.37	34.47	34.43	36.05	35.95	0.438	0.003[d,e]	0.728	0.983
NDF	60.01	59.19	59.55	59.68	60.68	60.33	0.552	0.227	0.408	0.681
NFC	12.40	13.33	15.20	14.57	19.63	18.12	0.570	< 0.001[c,d,e]	0.533	0.200
Starch	2.19	2.68	0.40	0.34	0.54	0.69	0.336	< 0.001[c,d]	0.293	0.575
ESC	5.74	5.16	7.11	6.94	8.31	8.49	0.438	< 0.001[c,d,e]	0.467	0.573
WSC	8.40	8.18	9.87	9.38	11.37	12.29	0.563	< 0.001[d,e]	0.868	0.380
CP	15.73	15.55	15.32	15.77	10.47[x]	11.42[b,x]	0.312	< 0.001[d,e]	0.020	0.061
Ash	8.96[x]	8.72[x]	8.05[y]	8.04[y]	6.27	6.99[b]	0.192	< 0.001[c,d,e]	0.244	0.019
Crude Fat	2.87	3.06	1.88	1.94	2.95	3.15	0.094	< 0.001[c,e]	0.037	0.699
Initial HM DM[f]	0.32	0.32	0.14	0.14	0.28	0.28	0.015	< 0.001[c,e]	0.864	0.918

[a] "Period" refers to the month in which the experiment was conducted (Period 1 = October, Period 2 = February, Period 3 = May). Cells refer to the 5m^2 adjacent enclosures used to estimate DMI in EXPT1. Horses grazed in cell A and B for two consecutive 4-h periods. Values for each cell are the mean of 8 individual cells for either cell A or B. Individual values for each of the 8 cells (A and B) were calculated as the mean of two samples collected immediately prior to and after grazing. Cells contained tall fescue (Max-Q, Pennington Seed, Madison, GA).

[b] Different superscripts within a row differ $P < 0.05$ between cells (Cell A differs from Cell B) within a period.

[c] Period 1 mean differs from period 2 mean

[d] Period 1 mean differs from period 3 mean

[e] Period 2 mean differs from period 3 mean

[f] HM DM for each cell is defined as the forage density of each cell (kg DM/m2), prior to grazing.

[x,y] Different superscripts within a row differ $P < 0.05$ across periods within a cell (Cell A-period 1 differs from Cell A period 3)

Table 3. Effect of period and cell on mean hourly DMI for EXPT1, periods 1, 2, 3[a,b]

	Cell A	Cell B	Mean	SE
Period 1	0.22[c,x]	0.11[x]	0.17[x]	0.0132
Period 2	0.10	0.08	0.09	0.0132
Period 3	0.12	0.09	0.11	0.0123

[a] "Period" refers to the month in which the experiment was conducted (Period 1 = October, Period 2 = February, Period 3 = May). Cells refer to the $5m^2$ adjacent enclosures used to estimate DMI in EXPT1. Horses grazed in cell A and B for two consecutive 4-h periods.
[b] Main effects: Period ($P < 0.001$), Cell ($P < 0.001$), Period x Cell ($P = 0.015$)
[c] Different superscripts within a row differ $P < 0.05$ between cells.
[x] Means within a column with different superscripts differ $P < 0.05$

Table 4. Experimental details for EXPT2 in periods 1, 2, and 3.

	Period 1	Period 2	Period 3
Start date	October 28, 2008	February 25, 2009	May 20, 2009
End date	December 10, 2008	March 31, 2009	July 1, 2009
Number of pastures	2	3	2
Time in pasture 1 (days)	21	13	21
Time in pasture 2 (days)	21	11	21
Time in pasture 3 (days)	NA	11	NA
Total days on pasture	42	35	42
Mean ambient temperature (ºC)	9.5 ± 0.5º	9.2 ± 2.3º	23.2 ± 2.9º
Initial HM DM pasture 1 (kg DM/m^2)	0.397	0.184	0.268
Initial HM DM pasture 2 (kg DM/m^2)	0.436	0.149	0.458
Initial HM DM pasture 3 (kg DM/m^2)	NA	0.179	NA
Mean BW (all horses)	576 (532 – 626)	527 (446.5 – 601)	557 (460.5 – 628)
Mean DE requirement (Mcal/day)	19.20 (17.8 – 20.9)	17.55 (14.9 – 20.0)	18.54 (15.3 – 20.9)
Hourly DMI rate kgDM·100 $kgBW^{-1}$· h^{-1}	0.166	0.088	0.108
DE of pastures (Mcal/kg)	2.25	2.09	2.04
Mean grazing time allotted (hrs)	9	18	14.5

Table 5. Chemical composition of tall fescue in Pasture 1 and 2 of EXPT2, period 1[a,b,c,d]

	Period 1							
	Pasture 1		Pasture 2			Main Effects *P*-values		
Item	UNRES	RES	UNRES	RES	SE	Treatment	Pasture	Treatment x Pasture
DM	33.46	31.93	41.01	38.52	0.867	0.046	< 0.001	0.624
DE	2.19	2.18	2.22	2.28	0.024	0.400	0.019	0.169
ADF	34.83	34.25	34.34	33.62	0.430	0.136	0.281	0.890
NDF	58.29	57.10	58.41	57.19	0.448	0.047	0.802	0.977
NFC	15.85	16.31	19.26	20.85	0.697	0.327	< 0.001	0.046
CP	15.23	15.51	13.46	13.02	0.499	0.895	0.001	0.381
Crude Fat	2.41	2.47	2.10	2.15	0.050	0.235	0.002	0.885
Ash	8.30	8.66	6.77	6.88	0.349	0.630	< 0.001	0.429
Initial HM DM (kg DM/m^2)	0.39	0.41	0.44	0.44	0.436	0.681	0.138	0.630

[a] "Period" refers to the 42-d during which the experiment was conducted (Period 1 = October 28 to December 12, 2008)
[b] Pastures 1 and 2 were each sized 66m x 100m and were each divided into 8 individual enclosures sized 33m x 25m. Each enclosure within the pasture contained a single horse.
[c] Horses grazed each pasture for a period of 21 consecutive days
[d] Values represent the mean of 3 samples collected immediately before, during, and immediately following grazing in each pasture

Table 6. Chemical composition of tall fescue in Pasture 1, 2, and 3 of EXPT2, period 2 [a,b,c,d]

	Period 2									
	Pasture 1		Pasture 2		Pasture 3			Main Effects *P*-values		
Item	UNRES	RES	UNRES	RES	UNRES	RES	SE	Treatment	Pasture	Treatment x Pasture
DM	45.51	47.94	37.40	36.65	33.68	33.03	1.610	0.824	< 0.001[x,y,z]	0.504
DE	2.11	2.23	1.94	2.09	2.09	2.05	0.050	0.222	0.006[x,y]	0.060
ADF	33.21	34.04	35.30	33.59	34.17	33.43	1.184	0.679	0.680	0.442
NDF	56.97	59.10	56.59	56.57	59.04	57.94	1.504	0.786	0.455	0.580
NFC	17.03	15.41	10.79	10.30	10.92	11.36	1.183	0.658	< 0.001[x,y]	0.457
CP	15.25	14.27	17.98	19.19	18.39	18.77	1.083	0.836	0.004[x,y]	0.586
Crude Fat	2.16	2.21	2.55	2.65	2.27	2.22	0.102	0.757	< 0.001[x,z]	0.626
Ash	8.59	9.02	12.09	11.56	9.38	9.72	0.781	0.934	< 0.001[x,z]	0.667
Initial HM DM (kg DM/m^2)	0.17	0.20	0.15	0.15	0.17	0.19	0.022	0.526	0.258	0.835

[a] "Period" refers to the 35-d during which the experiment was conducted (Period 2 = February 25 to April 24, 2009).
[b] Pasture 1 was sized 34m x 68m and was divided into 8 individual enclosures sized 17m x 34m. Pastures 2 and 3 were each sized 66m x 100m and were each divided into 8 individual enclosures sized 33m x 25m. Each enclosure within the pasture contained a single horse.
[c] Horses grazed pasture 1 for a period of 13 days and pastures 2 and 3 for 11 days, consecutively
[d] Values represent the mean of 2 samples collected immediately before, and immediately following grazing in each pasture
[x] Pasture 1 mean differs from pasture 2 mean
[y] Pasture 1 mean differs from pasture 3 mean
[z] Pasture 2 mean differs from pasture 3 mean

Table 7. Chemical composition of tall fescue in Pasture 1 and 2 of EXPT2, period 3[a,b,c,d]

	Period 3							
	Pasture 1		Pasture 2			Main Effects *P*-values		
Item	UNRES	RES	UNRES	RES	SE	Treatment	Pasture	Treatment x Pasture
DM	32.28	32.52	34.00	35.57	1.734	0.483	0.117	0.490
DE	2.07	2.08	1.98	2.02	0.027	0.453	0.018	0.409
ADF	40.18	41.24	43.29	42.41	0.831	0.924	0.025	0.228
NDF	64.36	65.12	67.53	67.04	0.917	0.906	0.007	0.366
NFC	12.59	13.65	12.11	12.82	0.510	0.197	0.134	0.661
CP	11.85	10.69	10.37	10.41	0.549	0.364	0.143	0.295
Crude Fat	3.44	3.17	2.59	2.79	0.121	0.815	0.001	0.071
Ash	7.76	6.95	7.42	6.94	0.369	0.223	0.457	0.460
Initial HM DM (kg DM/m^2)	0.27	0.27	0.48	0.43	0.053	0.603	0.017	0.677

[a] "Period" refers to the 42-d during which the experiment was conducted (Period 3 = May 20 to July 1, 2009).
[b] Pastures 1 and 2 were each sized 66m x 100m and were each divided into 8 individual enclosures sized 33m x 25m. Each enclosure within the pasture contained a single horse.
[c] Horses grazed each pasture for a period of 21 consecutive days
[d] Values represent the mean of 2 samples collected immediately before and immediately following grazing in each pasture

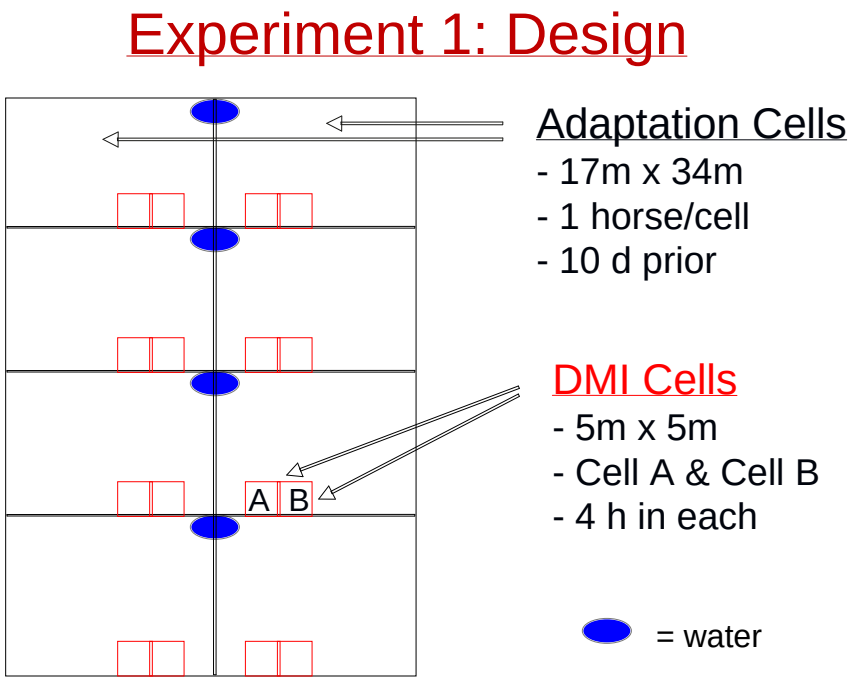

Figure 1. Diagram of Adaptation cells and DMI cells for EXPT1.

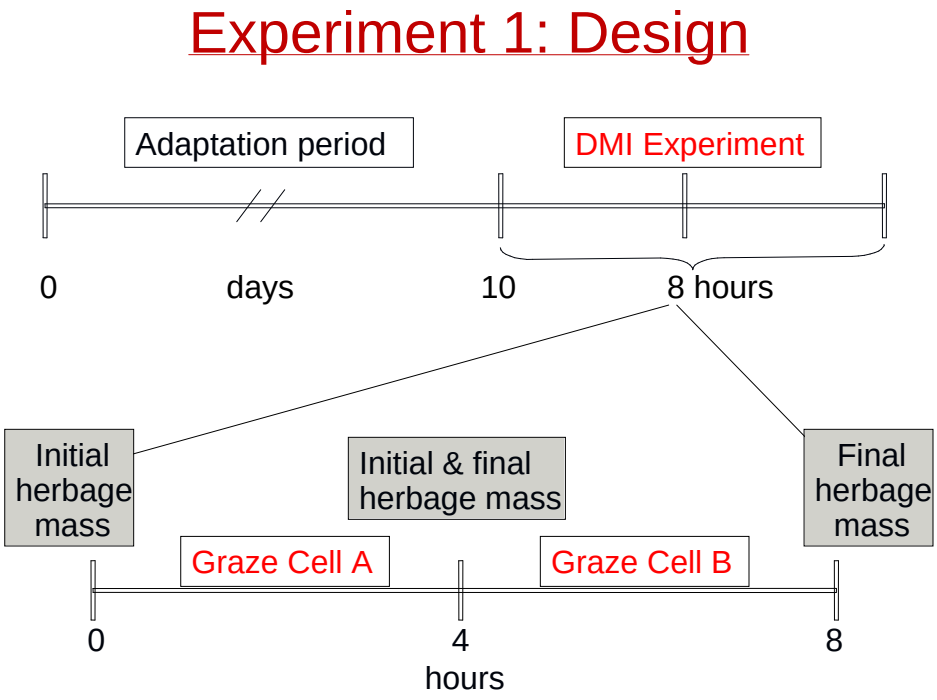

Figure 2. Diagram of the timeline for EXPT1.

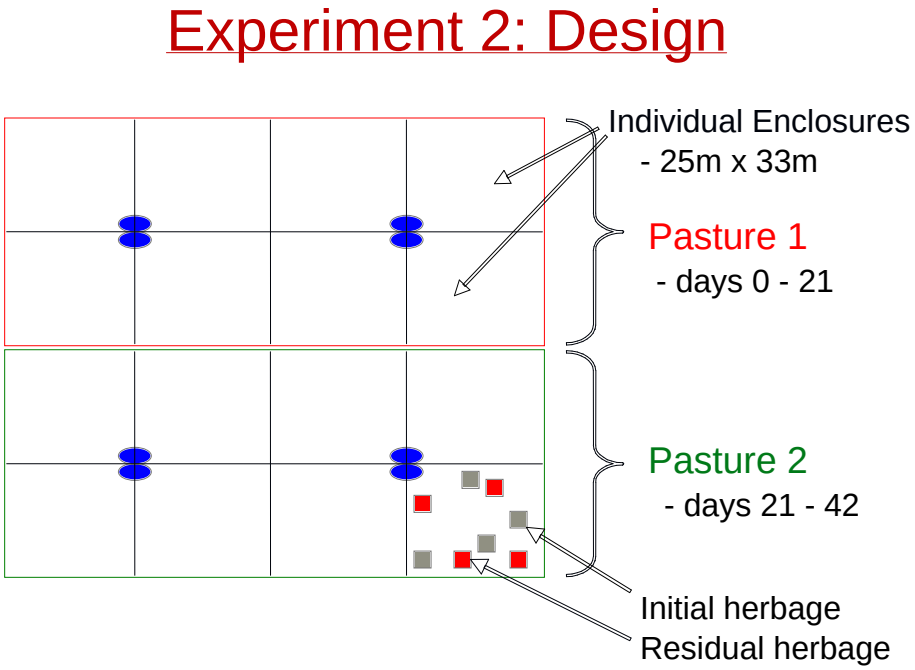

Figure 3. Diagram of pasture 1 and 2 in EXPT2.

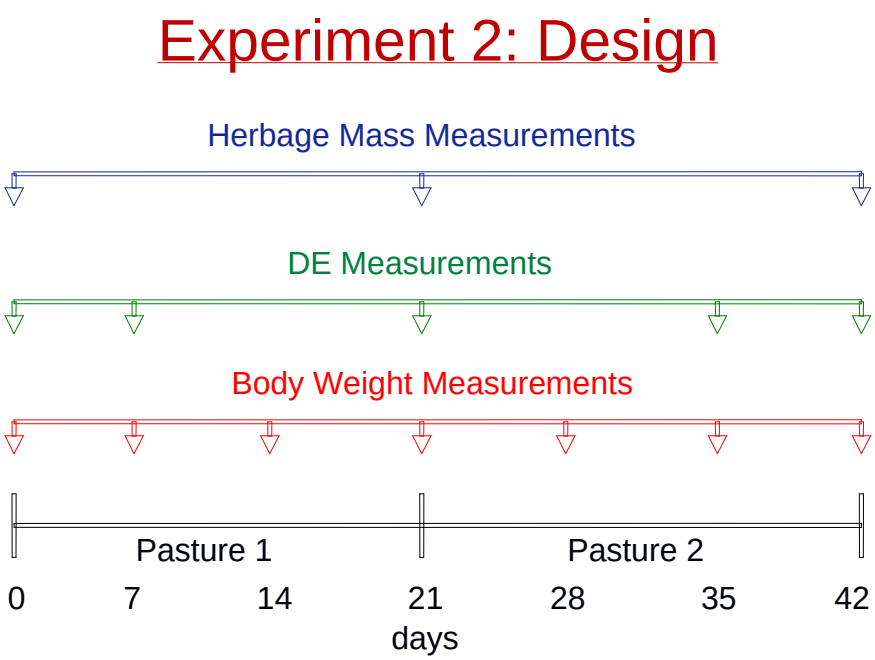

Figure 4. Experimental measurements taken during EXPT2 and the associated time of collection.

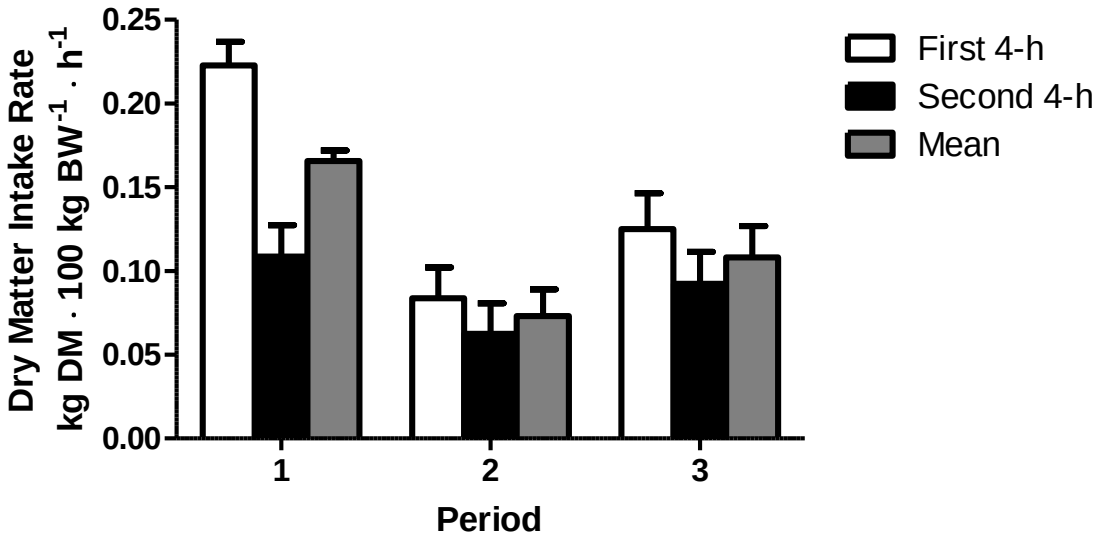

Figure 5. Hourly DMI rate of periods 1, 2, and 3. Period 1 (Oct.), Period 2 (Feb.), Period 3 (May). Hourly DMI rate over two consecutive 4-h periods for horses grazing tall fescue pasture (MaxQ®, Pennington Seed, Madison, GA) pasture. First 4-h vs. second 4-h ($P < 0.001$); Period (1 > 2 or 3; $P = 0.001$); Interaction (First 4-h > Second 4-h in period 1; $P = 0.015$).

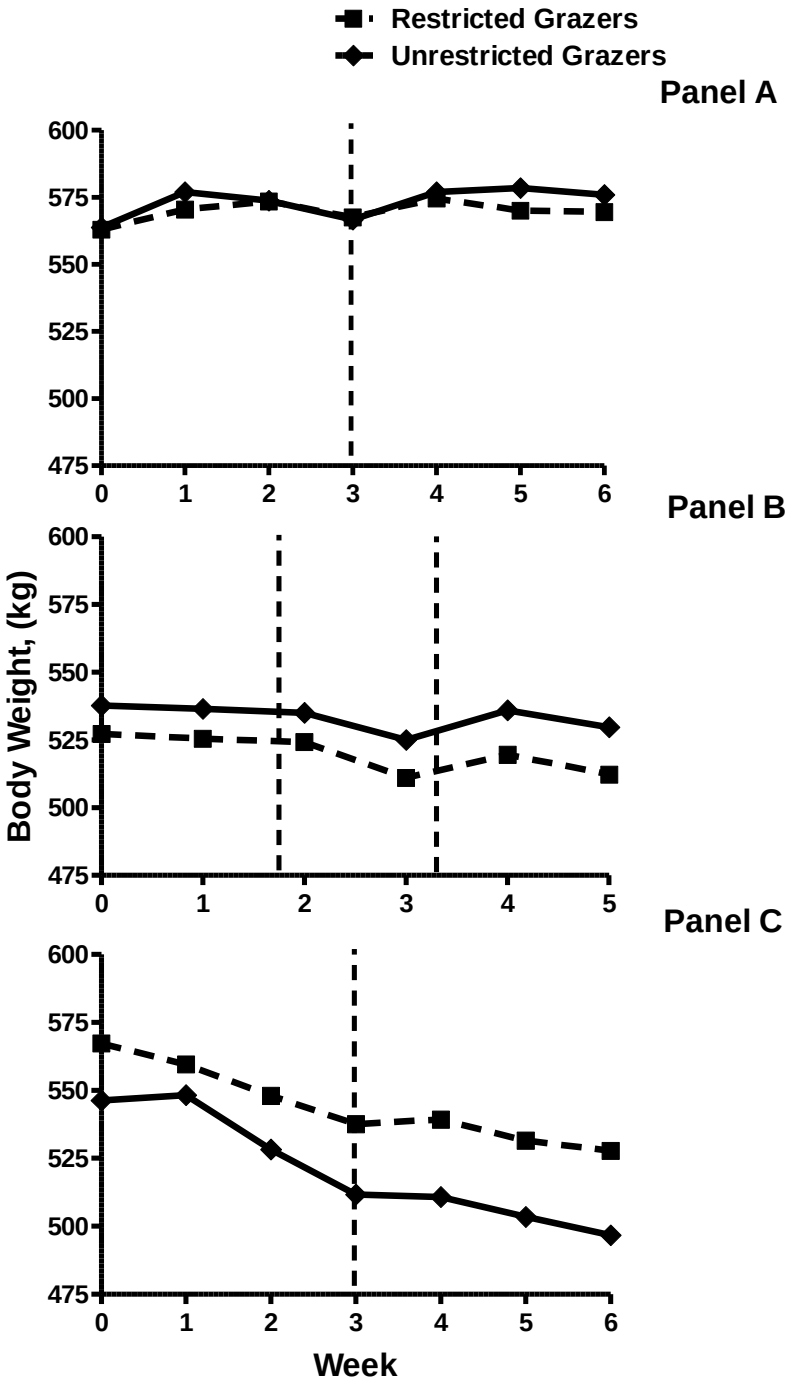

Figure 6. Changes in BW of RES and UNRES over time in EXPT2 period 1, 2, and 3. Period 1 (Oct. – Dec.) – panel A, Period 2 (Feb. – Apr.) – panel B, Period 3 (May – July) – panel C. Treatment (P = 0.95); Time (P = 0.02); Treatment x Time (P =0.86)

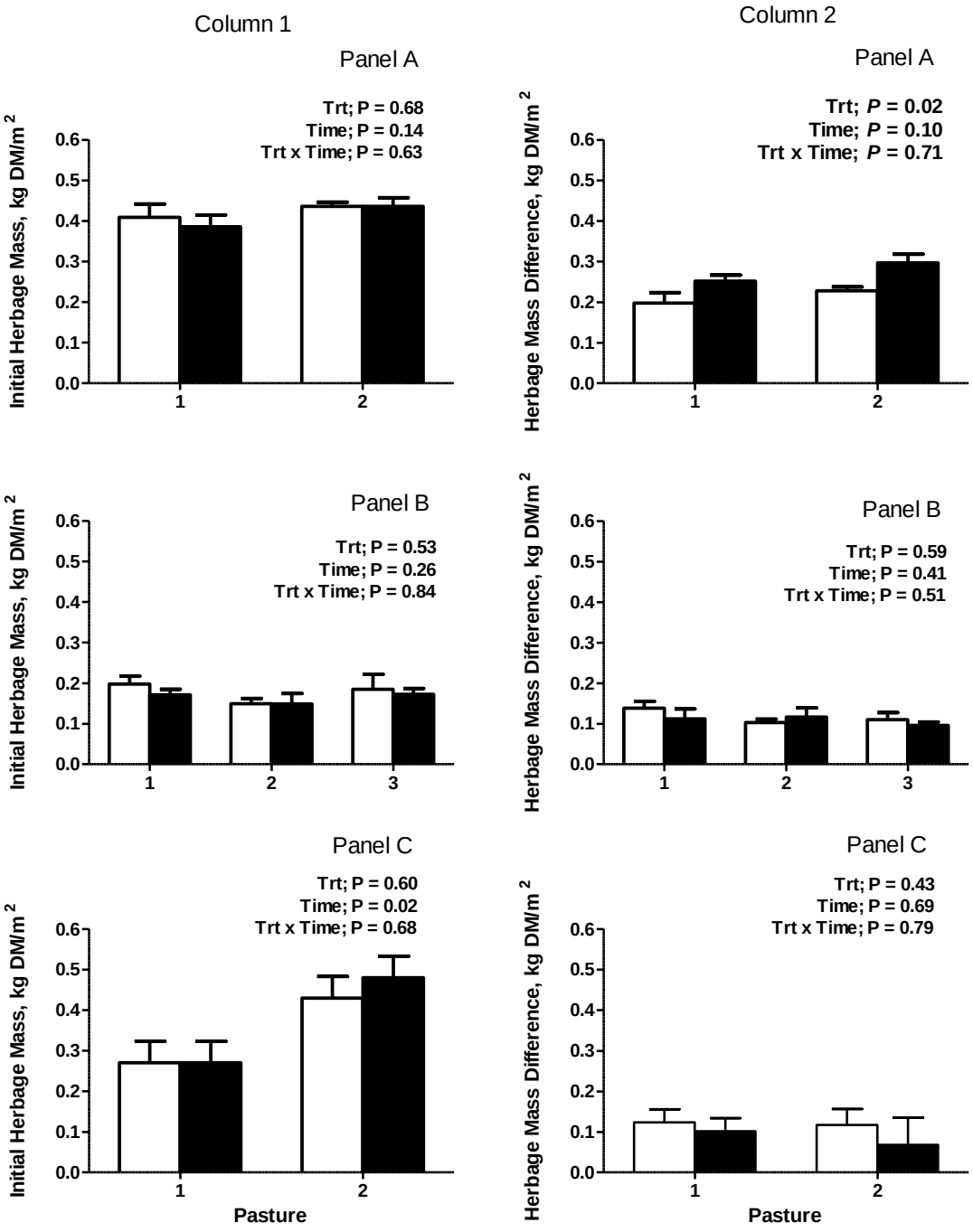

Figure 7. Initial HM DM and HM DM Difference in pastures of EXPT2 in periods 1, 2, and 3. Column 1 = Initial HM DM; Column 1 = HM DM Difference; Panel A – Period 1 (Oct. – Dec.); Panel B - Period 2 (Feb. – Apr.); Panel C - Period 3 (May – July).

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APPENDIX

Preliminary Experiments

In order to determine the most accurate method for estimating pasture dry matter intake, a number of preliminary experiments were conducted. These preliminary experiments included determining the best size sampling technique, including sample size, number of samples, sample location, and cell size. In order to determine the sample size which most accurately captured the forage mass within the $5m^2$ grazing cell, a $5m^2$ grazing cell was built in August of 2007. A 0.5 m x 0.5m wooden square was then used to collect samples within the cell. Ten random samples were cut, collected, weighed, and recorded. The remainder of the entire $5m^2$ cell was then cut, collected, and weighed to determine the total forage mass within the cell. The weights of the ten random samples were then used to predict the total yield of the $5m^2$ plot. This method over-predicted the total forage of the $5m^2$ cell. It was postulated that one potential reason for the over-prediction of the forage mass in the entire cell was the small size of the sampling square. With a 0.5m x 0.5m sampling square, the $5m^2$ cell was provided 100 samples, so 10 samples was a relatively small forage mass from which we were hoping to predict the mass of the entire cell.

The following preliminary study was conducted in August of 2007. For this experiment, a separate $5m^2$ cell was built in close proximity to the one used in the prior experiment. A new sampling square, sized 1m x 1m was then used to indicate 12 random samples within the cell. Samples were cut to below grazing height (~3 cm), collected, and weighed individually. The entire $5m^2$ cell was then cut, collected and weighed to determine the total forage mass within the cell. Dry matter was determined on composite samples of 3 random samples. It was

determined that 3 samples accurately represented the total forage mass within the entire cell. However, for the sake of increased accuracy, 4 samples were used during the DMI component of the trial.

Experimental Data

Appendix Table 1. Experiment 1 hourly dry matter intake estimates (kgDM/100kgBW/hr) for individual horses within periods and cells.

Horse	Period	Cell	DMI
Sinful	1	1	0.21
Sinful	1	2	0.12
Sinful	2	1	0.054
Sinful	2	2	0.082
Sinful	3	1	0.119
Sinful	3	2	0.067
Suzie	1	1	0.24
Suzie	1	2	0.07
Suzie	2	1	0.115
Suzie	2	2	0.090
Suzie	3	1	0.060
Suzie	3	2	0.039
Lucy	1	1	0.2
Lucy	1	2	0.1
Lucy	2	1	0.065
Lucy	2	2	0.107
Lucy	3	1	0.102
Lucy	3	2	0.152
ADT	1	1	0.17
ADT	1	2	0.15
ADT	2	1	0.094
ADT	2	2	0.075
ADT	3	1	0.258
ADT	3	2	0.189
Tex	1	1	
Tex	1	2	
Tex	2	1	0.156
Tex	2	2	0.058
Tex	3	1	0.093
Tex	3	2	0.035
Dee	1	1	0.23
Dee	1	2	0.16
Dee	2	1	-0.003

Dee	2	2	-0.056
Dee	3	1	0.147
Dee	3	2	0.064
Maile	1	1	0.29
Maile	1	2	0.02
Maile	2	1	0.139
Maile	2	2	0.076
Maile	3	1	0.092
Maile	3	2	0.109
Avalon	1	1	0.22
Avalon	1	2	0.14
Avalon	2	1	0.047
Avalon	2	2	0.072
Avalon	3	1	0.125
Avalon	3	2	0.080

Appendix Table 2. Experiment 2 – period 1 (Oct. – Dec.) body weight (kg) of individual horses within treatment and time.

Horse	Treatment	Week	BW
Sinful	UNRES	0	548
Sinful	UNRES	1	570
Sinful	UNRES	2	566
Sinful	UNRES	3	566
Sinful	UNRES	4	574
Sinful	UNRES	5	576
Sinful	UNRES	6	568
Suzie	RES	0	518
Suzie	RES	1	520
Suzie	RES	2	524
Suzie	RES	3	526
Suzie	RES	4	524
Suzie	RES	5	522
Suzie	RES	6	522
Lucy	UNRES	0	632
Lucy	UNRES	1	652
Lucy	UNRES	2	648
Lucy	UNRES	3	638
Lucy	UNRES	4	656
Lucy	UNRES	5	650
Lucy	UNRES	6	658
ADT	UNRES	0	497
ADT	UNRES	1	500
ADT	UNRES	2	495
ADT	UNRES	3	487
ADT	UNRES	4	502
ADT	UNRES	5	506
ADT	UNRES	6	494
Tex	RES	0	604
Tex	RES	1	606
Tex	RES	2	608
Tex	RES	3	602
Tex	RES	4	616
Tex	RES	5	602
Tex	RES	6	608
Dee	RES	0	552
Dee	RES	1	570

Dee	RES	2	576
Dee	RES	3	566
Dee	RES	4	582
Dee	RES	5	574
Dee	RES	6	564
Maile	UNRES	0	562
Maile	UNRES	1	548
Maile	UNRES	2	564
Maile	UNRES	3	562
Maile	UNRES	4	554
Maile	UNRES	5	562
Maile	UNRES	6	556
Avalon	RES	0	578
Avalon	RES	1	586
Avalon	RES	2	586
Avalon	RES	3	576
Avalon	RES	4	576
Avalon	RES	5	582
Avalon	RES	6	584

Appendix Table 3. Experiment 2 – period 2 (Feb. – Apr.) body weight (kg) of individual horses within treatment and time.

Horse	Treatment	Week	BW
Sinful	UNRES	0	506
Sinful	UNRES	1	502
Sinful	UNRES	2	502
Sinful	UNRES	3	497
Sinful	UNRES	4	502
Sinful	UNRES	5	502
Suzie	RES	0	510
Suzie	RES	1	502
Suzie	RES	2	506
Suzie	RES	3	491
Suzie	RES	4	506
Suzie	RES	5	498
Lucy	RES	0	606
Lucy	RES	1	600
Lucy	RES	2	602
Lucy	RES	3	580
Lucy	RES	4	592
Lucy	RES	5	578
ADT	RES	0	461
ADT	RES	1	456
ADT	RES	2	455
ADT	RES	3	445
ADT	RES	4	452
ADT	RES	5	449
Tex	UNRES	0	585
Tex	UNRES	1	582
Tex	UNRES	2	582
Tex	UNRES	3	575
Tex	UNRES	4	590
Tex	UNRES	5	578
Dee	RES	0	532
Dee	RES	1	544
Dee	RES	2	534
Dee	RES	3	528
Dee	RES	4	528
Dee	RES	5	524
Maile	UNRES	0	506

Maile	UNRES	1	508
Maile	UNRES	2	506
Maile	UNRES	3	496
Maile	UNRES	4	510
Maile	UNRES	5	509
Avalon	UNRES	0	554
Avalon	UNRES	1	554
Avalon	UNRES	2	550
Avalon	UNRES	3	532
Avalon	UNRES	4	542
Avalon	UNRES	5	530

Appendix Table 4. Experiment 2 – period 3 (May – July) body weight (kg) of individual horses within treatment and time.

Horse	Treatment	Week	BW
Sinful	UNRES	0	532
Sinful	UNRES	1	520
Sinful	UNRES	2	504
Sinful	UNRES	3	491
Sinful	UNRES	4	479
Sinful	UNRES	5	474
Sinful	UNRES	6	468
Suzie	RES	0	538
Suzie	RES	1	534
Suzie	RES	2	522
Suzie	RES	3	502
Suzie	RES	4	504
Suzie	RES	5	494
Suzie	RES	6	483
Lucy	UNRES	0	628
Lucy	UNRES	1	632
Lucy	UNRES	2	616
Lucy	UNRES	3	600
Lucy	UNRES	4	604
Lucy	UNRES	5	600
Lucy	UNRES	6	592
ADT	UNRES	0	460.5
ADT	UNRES	1	437
ADT	UNRES	2	439
ADT	UNRES	3	426
ADT	UNRES	4	422
ADT	UNRES	5	412
ADT	UNRES	6	409
Tex	RES	0	610
Tex	RES	1	604
Tex	RES	2	592
Tex	RES	3	586
Tex	RES	4	586
Tex	RES	5	580
Tex	RES	6	574
Dee	UNRES	0	564
Dee	UNRES	1	604

Dee	UNRES	2	554
Dee	UNRES	3	530
Dee	UNRES	4	538
Dee	UNRES	5	528
Dee	UNRES	6	518
Maile	RES	0	539
Maile	RES	1	536
Maile	RES	2	524
Maile	RES	3	516
Maile	RES	4	514
Maile	RES	5	510
Maile	RES	6	506
Avalon	RES	0	582
Avalon	RES	1	564
Avalon	RES	2	554
Avalon	RES	3	546
Avalon	RES	4	553
Avalon	RES	5	542
Avalon	RES	6	548

Appendix Table 5. Experiment 2 – period 1 (Oct. – Dec.) digestible energy (Mcal/kg) content of forage within treatment and pasture.

Horse	Treatment	Pasture	Time	DE
ADT	UNRES	1	0	2.3398
ADT	UNRES	1	12	2.2044
ADT	UNRES	1	24	2.0369
ADT	UNRES	2	0	2.4211
ADT	UNRES	2	12	2.2796
ADT	UNRES	2	24	2.1136
Avalon	RES	1	0	2.1812
Avalon	RES	1	12	2.2167
Avalon	RES	1	24	2.1957
Avalon	RES	2	0	2.4003
Avalon	RES	2	12	2.33887
Avalon	RES	2	24	2.1907
Dee	RES	1	0	2.3705
Dee	RES	1	12	2.165
Dee	RES	1	24	1.8861
Dee	RES	2	0	2.3822
Dee	RES	2	12	2.2583
Dee	RES	2	24	2.1053
Lucy	UNRES	1	0	2.4313
Lucy	UNRES	1	12	2.2663
Lucy	UNRES	1	24	2.0179
Lucy	UNRES	2	0	2.3463
Lucy	UNRES	2	12	2.1806
Lucy	UNRES	2	24	1.9367
Maile	UNRES	1	0	2.3081
Maile	UNRES	1	12	2.2227
Maile	UNRES	1	24	1.9779
Maile	UNRES	2	0	2.3416
Maile	UNRES	2	12	2.2274
Maile	UNRES	2	24	2.0538
Sinful	UNRES	1	0	2.292
Sinful	UNRES	1	12	2.3143
Sinful	UNRES	1	24	1.8572
Sinful	UNRES	2	0	2.4146
Sinful	UNRES	2	12	2.3182

Sinful	UNRES	2	24	2.0401
Suzie	RES	1	0	2.3689
Suzie	RES	1	12	2.2815
Suzie	RES	1	24	2.0816
Suzie	RES	2	0	2.4633
Suzie	RES	2	12	2.3785
Suzie	RES	2	24	2.1669
Tex	RES	1	0	2.3378
Tex	RES	1	12	2.2203
Tex	RES	1	24	1.8637
Tex	RES	2	0	2.4033
Tex	RES	2	12	2.1866
Tex	RES	2	24	2.0858

Appendix Table 6. Experiment 2 – period 2 (Feb. – Apr.) digestible energy (Mcal/kg) content of forage within treatment and pasture.

Horse	Treatment	Pasture	Time	DE
ADT	RES	1	0	2.118
ADT	RES	1	12	1.82
ADT	RES	2	0	2.145
ADT	RES	2	12	1.56
ADT	RES	3	0	2.1
ADT	RES	3	12	1.87
Avalon	UNRES	1	0	2.33
Avalon	UNRES	1	12	2.087
Avalon	UNRES	2	0	2.236
Avalon	UNRES	2	12	2.116
Avalon	UNRES	3	0	2.22
Avalon	UNRES	3	12	1.97
Dee	RES	1	0	2.215
Dee	RES	1	12	2.185
Dee	RES	2	0	2.305
Dee	RES	2	12	1.79
Dee	RES	3	0	2.11
Dee	RES	3	12	1.92
Lucy	RES	1	0	2.26
Lucy	RES	1	12	2.019
Lucy	RES	2	0	2.182
Lucy	RES	2	12	1.987
Lucy	RES	3	0	2.17
Lucy	RES	3	12	1.99
Maile	UNRES	1	0	2.27
Maile	UNRES	1	12	1.947
Maile	UNRES	2	0	2.176
Maile	UNRES	2	12	1.67
Maile	UNRES	3	0	2.11
Maile	UNRES	3	12	1.87
Sinful	UNRES	1	0	2.38
Sinful	UNRES	1	12	2.38
Sinful	UNRES	2	0	2.181
Sinful	UNRES	2	12	1.96
Sinful	UNRES	3	0	2.157

Sinful	UNRES	3	12	1.9
Suzie	RES	1	0	2.36
Suzie	RES	1	12	1.975
Suzie	RES	2	0	2.19
Suzie	RES	2	12	2.12
Suzie	RES	3	0	2.34
Suzie	RES	3	12	2.13
Tex	UNRES	1	0	2.35
Tex	UNRES	1	12	2.03
Tex	UNRES	2	0	2.107
Tex	UNRES	2	12	1.619
Tex	UNRES	3	0	2.21
Tex	UNRES	3	12	2.116

Appendix Table 7. Experiment 2 – period 3 (May – July) digestible energy (Mcal/kg) content of forage within treatment and pasture.

Horse	Treatment	Pasture	Time	DE
Sinful	UNRES	1	0	2.119
Sinful	UNRES	1	21	2.035
Sinful	UNRES	2	0	2.06
Sinful	UNRES	2	21	1.755
Suzie	RES	1	0	2.02
Suzie	RES	1	21	1.97
Suzie	RES	2	0	2.109
Suzie	RES	2	21	1.89
Lucy	UNRES	1	0	2.108
Lucy	UNRES	1	21	2.14
Lucy	UNRES	2	0	2.087
Lucy	UNRES	2	21	1.968
ADT	UNRES	1	0	1.98
ADT	UNRES	1	21	2.026
ADT	UNRES	2	0	2.14
ADT	UNRES	2	21	1.855
Tex	RES	1	0	2.068
Tex	RES	1	21	2.08
Tex	RES	2	0	2.124
Tex	RES	2	21	1.81
Dee	UNRES	1	0	2.095
Dee	UNRES	1	21	2.049
Dee	UNRES	2	0	2.07
Dee	UNRES	2	21	1.906
Maile	RES	1	0	2.202
Maile	RES	1	21	2.029
Maile	RES	2	0	2.129
Maile	RES	2	21	1.93
Avalon	RES	1	0	2.134
Avalon	RES	1	21	2.099
Avalon	RES	2	0	2.237
Avalon	RES	2	21	1.968

Appendix Table 8. Experiment 2 – period 1 (Oct. – Dec.) dry matter (%) content of forage within treatment and pasture.

Horse	Treatment	Pasture	Time	DM
Sinful	UNRES	1	0	25.16
Sinful	UNRES	1	12	21.64
Sinful	UNRES	1	24	53.43
Sinful	UNRES	2	0	33.82
Sinful	UNRES	2	12	46.41
Sinful	UNRES	2	24	55.63
Suzie	RES	1	0	27.98
Suzie	RES	1	12	24.59
Suzie	RES	1	24	43.61
Suzie	RES	2	0	31.98
Suzie	RES	2	12	38.75
Suzie	RES	2	24	49.94
Lucy	UNRES	1	0	25.57
Lucy	UNRES	1	12	27.04
Lucy	UNRES	1	24	50.19
Lucy	UNRES	2	0	33.13
Lucy	UNRES	2	12	37.81
Lucy	UNRES	2	24	45.32
ADT	UNRES	1	0	25.64
ADT	UNRES	1	12	25.59
ADT	UNRES	1	24	49.63
ADT	UNRES	2	0	30.65
ADT	UNRES	2	12	40.25
ADT	UNRES	2	24	47.09
Tex	RES	1	0	22.09
Tex	RES	1	12	23.01
Tex	RES	1	24	52.32
Tex	RES	2	0	31.05
Tex	RES	2	12	40.62
Tex	RES	2	24	44.16
Dee	RES	1	0	22.54
Dee	RES	1	12	24.07
Dee	RES	1	24	51.19
Dee	RES	2	0	29.34
Dee	RES	2	12	36.79

Dee	RES	2	24	44.56
Maile	UNRES	1	0	26.81
Maile	UNRES	1	12	26.95
Maile	UNRES	1	24	43.86
Maile	UNRES	2	0	30.27
Maile	UNRES	2	12	39.01
Maile	UNRES	2	24	52.67
Avalon	RES	1	0	27.78
Avalon	RES	1	12	25.65
Avalon	RES	1	24	38.29
Avalon	RES	2	0	34.29
Avalon	RES	2	12	35.2
Avalon	RES	2	24	45.52

Appendix Table 9. Experiment 2 – period 2 (Feb. – Apr.) dry matter (%) content of forage within treatment and pasture.

Horse	Treatment	Pasture	Time	DM
Sinful	UNRES	1	0	40.94
Sinful	UNRES	1	12	55.86
Sinful	UNRES	2	0	42.53
Sinful	UNRES	2	12	35.97
Sinful	UNRES	3	0	36.6
Sinful	UNRES	3	12	28.48
Suzie	RES	1	0	40.66
Suzie	RES	1	12	51.37
Suzie	RES	2	0	36.93
Suzie	RES	2	12	32.76
Suzie	RES	3	0	31.24
Suzie	RES	3	12	28.83
Lucy	RES	1	0	44.63
Lucy	RES	1	12	47.81
Lucy	RES	2	0	42.13
Lucy	RES	2	12	32.07
Lucy	RES	3	0	33.69
Lucy	RES	3	12	29.58
ADT	RES	1	0	43.04
ADT	RES	1	12	60.18
ADT	RES	2	0	38.27
ADT	RES	2	12	30.89
ADT	RES	3	0	36.89
ADT	RES	3	12	29.22
Tex	UNRES	1	0	44.35
Tex	UNRES	1	12	48.04
Tex	UNRES	2	0	48.09
Tex	UNRES	2	12	40.06
Tex	UNRES	3	0	33.85
Tex	UNRES	3	12	31.12
Dee	RES	1	0	42.38
Dee	RES	1	12	53.43
Dee	RES	2	0	39.8
Dee	RES	2	12	40.31
Dee	RES	3	0	39.81

Dee	RES	3	12	35
Maile	UNRES	1	0	39.08
Maile	UNRES	1	12	43.76
Maile	UNRES	2	0	37.67
Maile	UNRES	2	12	28.13
Maile	UNRES	3	0	37.66
Maile	UNRES	3	12	30.45
Avalon	UNRES	1	0	45.07
Avalon	UNRES	1	12	46.96
Avalon	UNRES	2	0	40.7
Avalon	UNRES	2	12	26.03
Avalon	UNRES	3	0	35.23
Avalon	UNRES	3	12	36.03

Appendix Table 10. Experiment 2 – period 3 (May – July) dry matter (%) content of forage within treatment and pasture.

Horse	Treatment	Pasture	Time	DM
Sinful	UNRES	1	0	25.65
Sinful	UNRES	1	21	38.43
Sinful	UNRES	2	0	26.72
Sinful	UNRES	2	21	27.38
Suzie	RES	1	0	24.49
Suzie	RES	1	21	40.09
Suzie	RES	2	0	38.8
Suzie	RES	2	21	43.62
Lucy	UNRES	1	0	22.69
Lucy	UNRES	1	21	35.16
Lucy	UNRES	2	0	37.04
Lucy	UNRES	2	21	33.35
ADT	UNRES	1	0	22.27
ADT	UNRES	1	21	46.21
ADT	UNRES	2	0	33.69
ADT	UNRES	2	21	36.97
Tex	RES	1	0	25.42
Tex	RES	1	21	34.57
Tex	RES	2	0	35.57
Tex	RES	2	21	33.69
Dee	UNRES	1	0	27.46
Dee	UNRES	1	21	40.33
Dee	UNRES	2	0	39.01
Dee	UNRES	2	21	37.87
Maile	RES	1	0	26.03
Maile	RES	1	21	43.64
Maile	RES	2	0	38.66
Maile	RES	2	21	37.13
Avalon	RES	1	0	26.6
Avalon	RES	1	21	39.34
Avalon	RES	2	0	30.86
Avalon	RES	2	21	34.21

Appendix Table 11. Experiment 2 – period 1 (Oct. – Dec.) initial herbage mass estimates (kg DM/m^2) for pasture 1 or 2 within treatment.

Horse	Treatment	Pasture	Time	Yield
Sinful	UNRES	1	0	0.320
Sinful	UNRES	2	21	0.399
Suzie	RES	1	0	0.327
Suzie	RES	2	21	0.409
Lucy	UNRES	1	0	0.355
Lucy	UNRES	2	21	0.498
ADT	UNRES	1	0	0.426
ADT	UNRES	2	21	0.428
Tex	RES	1	0	0.465
Tex	RES	2	21	0.460
Dee	RES	1	0	0.386
Dee	RES	2	21	0.442
Maile	UNRES	1	0	0.441
Maile	UNRES	2	21	0.418
Avalon	RES	1	0	0.457
Avalon	RES	2	21	0.432

Appendix Table 12. Experiment 2 – period 2 (Feb. – Apr.) initial herbage mass estimates (kg DM/m^2) for pasture 1, 2, or 3 within treatment.

Horse	Treatment	Pasture	Time	Yield
Sinful	UNRES	1	0	0.156
Sinful	UNRES	2	12	0.151
Sinful	UNRES	3	24	0.183
Suzie	RES	1	0	0.224
Suzie	RES	2	12	0.130
Suzie	RES	3	24	0.082
Lucy	RES	1	0	0.177
Lucy	RES	2	12	0.137
Lucy	RES	3	24	0.185
ADT	RES	1	0	0.151
ADT	RES	2	12	0.145
ADT	RES	3	24	0.251
Tex	UNRES	1	0	0.181
Tex	UNRES	2	12	0.213
Tex	UNRES	3	24	0.144
Dee	RES	1	0	0.236
Dee	RES	2	12	0.185
Dee	RES	3	24	0.220
Maile	UNRES	1	0	0.205
Maile	UNRES	2	12	0.143
Maile	UNRES	3	24	0.208
Avalon	UNRES	1	0	0.141
Avalon	UNRES	2	12	0.089
Avalon	UNRES	3	24	0.155

Appendix Table 13. Experiment 2 – period 3 (May – July) initial herbage mass estimates (kg DM/m^2) for pasture 1 or 2 within treatment.

Horse	Treatment	Pasture	Time	Yield
Sinful	UNRES	1	0	0.300
Sinful	UNRES	2	21	0.692
Suzie	RES	1	0	0.239
Suzie	RES	2	21	0.382
Lucy	UNRES	1	0	0.379
Lucy	UNRES	2	21	0.305
ADT	UNRES	1	0	0.169
ADT	UNRES	2	21	0.473
Tex	RES	1	0	0.264
Tex	RES	2	21	0.559
Dee	UNRES	1	0	0.222
Dee	UNRES	2	21	0.463
Maile	RES	1	0	0.338
Maile	RES	2	21	0.357
Avalon	RES	1	0	0.231
Avalon	RES	2	21	0.432

Appendix Table 14. Experiment 2 – period 1 (Oct. – Dec.) difference in herbage mass (kg DM/m^2) between initial and final samples in each pasture 1 or 2 within treatment.

Horse	Treatment	Pasture	Yield diff.
Sinful	UNRES	1	0.236
Sinful	UNRES	2	0.288
Suzie	RES	1	0.123
Suzie	RES	2	0.210
Lucy	UNRES	1	0.248
Lucy	UNRES	2	0.356
ADT	UNRES	1	0.227
ADT	UNRES	2	0.292
Tex	RES	1	0.224
Tex	RES	2	0.212
Dee	RES	1	0.207
Dee	RES	2	0.243
Maile	UNRES	1	0.296
Maile	UNRES	2	0.250
Avalon	RES	1	0.236
Avalon	RES	2	0.247

Appendix Table 15. Experiment 2 – period 2 (Feb. – Apr.) difference in herbage mass (kg DM/m^2) between initial and final samples in each pasture 1, 2, or 3 within treatment.

Horse	Treatment	Pasture	Yield diff.
Sinful	UNRES	1	0.128
Sinful	UNRES	2	0.125
Sinful	UNRES	3	0.105
Suzie	RES	1	0.131
Suzie	RES	2	0.127
Suzie	RES	3	0.063
Lucy	RES	1	0.124
Lucy	RES	2	0.090
Lucy	RES	3	0.110
ADT	RES	1	0.110
ADT	RES	2	0.102
ADT	RES	3	0.150
Tex	UNRES	1	0.092
Tex	UNRES	2	0.172
Tex	UNRES	3	0.110
Dee	RES	1	0.188
Dee	RES	2	0.092
Dee	RES	3	0.115
Maile	UNRES	1	0.171
Maile	UNRES	2	0.107
Maile	UNRES	3	0.094
Avalon	UNRES	1	0.059
Avalon	UNRES	2	0.061
Avalon	UNRES	3	0.074

Appendix Table 16. Experiment 2 – period 3 (May – July) difference in herbage mass (kg DM/m^2) between initial and final samples in each pasture 1 or 2 within treatment.

Horse	Treatment	Pasture	Yield diff.
Sinful	UNRES	1	0.073
Sinful	UNRES	2	0.252
Suzie	RES	1	0.099
Suzie	RES	2	0.009
Lucy	UNRES	1	0.197
Lucy	UNRES	2	-0.067
ADT	UNRES	1	0.052
ADT	UNRES	2	0.070
Tex	RES	1	0.088
Tex	RES	2	0.134
Dee	UNRES	1	0.084
Dee	UNRES	2	0.016
Maile	RES	1	0.220
Maile	RES	2	0.202
Avalon	RES	1	0.087
Avalon	RES	2	0.122