

## ABSTACT

GILL, JENNIFER C. Weight Management in Horses: Relationships among Digestible Energy Intake, Body Weight, and Body Condition. (Under the direction of Dr. Shannon Pratt-Phillips and Dr. Paul Siciliano.)

Obesity can be associated with metabolic dysfunction and laminitis in horses with a genetic predisposition and the right environmental conditions. Experiment 1 (EXPT 1) was conducted to determine if owners could manage a feeding program that would result in body weight reduction in horses. Experiment 2 (EXPT 2) was conducted to determine the calorie intake level for horses fed to moderately increase in body condition. Experiment 3 (EXPT 3) investigated the factors affecting energy balance in horses by quantifying the within- and between-horse variability in pasture-associated activity and digestible energy intake in grazing horses. Experiment 4 (EXPT 4) was conducted to determine if body weight (BW), body condition score (BCS), and insulin (INS) concentration could be reduced when a restricted grazing protocol was implemented, limiting both time at pasture and space available for grazing.

In EXPT 1, twenty-four, overweight client-owned and managed horses were identified for weight reduction. After complete diet and health analyses, owners were given weight loss protocols to follow, consisting of the specific diet and management instructions to follow for  $26 \pm 4$  wks. Decreases ( $p < 0.01$ ) were observed in BW (31.48 kg, 95% CI: 24.07 to 38.89 kg), BCS (1.4/9, 95% CI: 1.01 to 1.80), cresty neck score (CNS; 0.96, 95% CI: 0.64 to 1.30), and INS (9.13  $\mu\text{U/mL}$ , 95% CI: 5.74 to 12.52). Owners that fully complied complied with the weight management protocols saw a greater

reduction ( $p < 0.01$ ) in their horses' BW and BCS than non-compliant owners. Likewise, horses with no pasture access had a greater mean decrease ( $p < 0.01$ ) in BW and BCS compared to horses having continual pasture access. Results suggest that 10% to 20% restriction of energy intake can be implemented in client-owned horses, resulting in a decrease in BW, BCS, and INS, if the owner reduces feed and pasture intake.

In EXPT 2, thirty-five Quarter Horse (age  $5.3 \pm 1.2$  yr; BW  $462 \pm 39$  kg; BCS  $4.5 \pm 0.5$ ) geldings were offered 1.75 to 2.00 kg DM/100 kg BW in mixed grass hay and 0.2 kg/100 kg BW in whole oats for a 42-d feeding trial. For each horse, the mean change in BW and BCS from d-0 to d-42 was evaluated using paired  $t$  tests. Trial 1 horses consumed  $53.21 \pm 0.58$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> and BW increased ( $p < 0.001$ ) by  $24 \pm 3$  kg; however, there was no ( $p = 0.43$ ) change in BCS ( $0.0 \pm 0.5$ ) from d 0 to d 42. When Trial 2 horses consumed  $47.68 \pm 2.10$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup>, BW increased ( $p = 0.009$ ) by  $8 \pm 3$  kg and BCS tended to increase ( $p = 0.06$ ) by  $0.5 \pm 0.5$  from 0 to 42 d. Trial 3 horses consumed  $51.22 \pm 2.19$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> and BW and BCS increased ( $p < 0.001$ ) by  $22 \pm 2$  kg and  $1.0 \pm 0.5$ , respectively, from d 0 to d 42. Despite considerable variability in calories consumed per kg of BW gain, the mean requirement was  $24 \pm 3$  Mcal per kg of BW gain, which was consistent with previous literature (NRC, 2007).

Experiment 3 (EXPT 3) was a preliminary study designed to test the variability in pasture-associated activity (heart rate (HR) and distance traveled) and estimate the digestible energy intake in grazing horses to better understand the factors affecting energy balance in horses. Eight mature, idle geldings (BW  $618 \pm 58$  kg; 5 to 12 years; BCS  $7.0 \pm 1.5$ ) were maintained continuously on a 7.7 acre non-toxic endophyte-infected tall fescue pasture for 12 days. Horses were subjected to two, 3-d collection

periods of heart rate (HR) monitoring/distance traveled (GPS) tracking using Polar V800 activity monitoring devices and total fecal collection using equine nappies. Daily fecal and pasture samples (1 per horse per day) were evaluated for acid insoluble ash (AIA), and the dry matter digestibility (DMD) was calculated as  $(1 - \text{AIA}_{\text{diet}} / \text{AIA}_{\text{feces}}) \times 100$ . The within-horse repeatability for heart rate (HR), distance traveled (DT), and fecal output (FO) was calculated as the within-horse standard deviation ( $S_w$ ), and the product of  $S_w$  and 2.77 was the repeatability coefficient (RC). Between horse differences were assessed using one-way analysis of variance. The AIA measurement in the feces was unsuccessful at determining the digestibility of pasture in grazing horses. Heart rate and FO were repeatable measures within-horses and between Group 1 and Group 2 (RC = 4 bpm and RC = 1.63 and 1.66 g·kg BW<sup>-1</sup>·d<sup>-1</sup>, respectively). Indices of HR and FO showed significant ( $p < 0.001$ ;  $p < 0.001$ ) between horse variability, which was likely due to differences in temperament, metabolism, and intake. Distance traveled was not consistent within horses or across days of collection.

In EXPT 4, ten mature, idle geldings were randomly assigned to either a RG group (BW 584 ± 70 kg, 5 to 12 years, n=5), where horses had access to pasture for 8 h/d and the grazing cell contained a herbage allowance to provide 80% of the mean NRC (2007) maintenance digestible energy (DE) requirement for 7 d, or a 24 h grazing group (CTRL; BW 571 ± 51 kg, 5 – 12 years, n=5) for 35 d. The RG group spent the remaining 15 h per day was in individual 3.7 m × 12.2 m dry lot pens with feed withheld. The CTRL horses had continuous access to pasture in excess of requirements. Weekly scale BWs and BCSs were recorded and venous blood samples were harvested for insulin concentration analysis prior to turnout. Differences in activity between

groups was assessed using Polar V800 activity monitoring devices in two, 3 d periods of collection that were exactly 2 wks apart. Weekly repeated measurements of BW, BCS, and INS concentration were analyzed using repeated measures ANOVA (PROC MIXED; autoregressive type 1 covariate and horse random effects) in SAS. The weekly herbage mass (HM) allowance and HM removal rate for RG horses was 11, 12, 10, 17, 14, and 32 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> and 71%, 40%, 71%, 59%, 66%, and 48%, respectively. Intake restriction resulting in a greater loss ( $p<0.001$ ) of BW and BCS over time than the CTRL horses. Restricted grazing horse lost  $35 \pm 4$  kg in BW and BCS decreased by  $1.0 \pm 0.5$  units. The CTRL horse increased in BW ( $14 \pm 4$  kg) and BCS ( $0.5 \pm 1.0$ ). Weekly INS was unaffected by the treatment group ( $p=0.52$ ) or the treatment by time interaction ( $p=0.71$ ), but was affected by time ( $p=0.12$ ) and appeared to mirror pasture non-structural carbohydrate content. Limiting time at pasture and herbage allowance resulted in a reduction in BW and BCS, while maximizing the utilization of available forage by promoting uniform grazing.

Weight Management in Horses: Relationships among Digestible Energy Intake, Body  
Weight, and Body Condition

by  
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## DEDICATION

This dissertation is dedicated to my church family, who have lifted me up in prayer countless times, and my family, friends, and mentors who have shaped me into the individual that I am today. I would like to thank my parents for always praying and supporting my decisions. I would like to thank my friends that love me despite my endless busyness. Looking back on my academic life, I remember the days of struggling through school because of my learning disability; I feel thankful for those Professors and support staff that saw my true potential and didn't give up on me. I can faithfully say that through perseverance and determination those with shortcomings can accomplish their dreams.

## BIOGRAPHY

Jennifer Christine Gill has a passion for horses than began at a young age when she first rode her Grandmother's Champion Dressage horse. Jennifer is originally from Bethlehem, Pennsylvania and when she was 13, she became the proud owner of a 3-year-old Paint gelding. Jennifer trained and showed in Western Pleasure and Hunter-Under-Saddle, under the guidance of Gale Remington, earning many local awards and a Register of Merit in Western Pleasure. After graduating high school, Jennifer moved to Westerville, Ohio and completed a Bachelor's degree in Equine Science from Otterbein University, where she concentrated in equine veterinary science. Because of her love for pathology, Jennifer worked part-time as an ER veterinary assistant while attending Universities. In 2012, Jennifer moved to Raleigh, North Carolina to pursue a Doctoral degree in Animal Science, specializing in equine nutrition. Jennifer's passion for helping horses and their owners influenced her decision to devote her spare time to being a nutritional consultant for private farms and speaking at extension meetings. Jennifer currently rides Hunter/Jumper horses at Finally Farm in Raleigh, North Carolina.

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## **CHAPTER 1: LITERATURE REVIEW**

### **1.1 Assessment of Equine Body Composition**

#### ***1.1 a. Evaluation of Equine Obesity***

The evaluation of body weight and body condition are important tools for developing rations that are appropriate for maintaining optimal health in horses. Fat deposition in horses targets specific areas of the body, including the crest of the neck, behind the withers, behind the shoulder, over the ribs, along the loins, and around the tailhead (Henneke et al. 1983). Visceral fat envelops internal organs and is located in peritoneal cavities and around body fossas' (Bianchi 1989). Equine body fat has been measured using the visual appraisal of the horse's body condition and measured quantitatively by B-mode ultrasound, total body water (TBW)/deuterium oxide dilution, bioelectrical impedance (BIA), and cadaver dissection (Kane et al. 1987, Kearns et al. 2002, Westervelt et al. 1976). Ultrasonic fat depth measures subcutaneous fat depth using ultrasound waves. This method can provide an effective measurement of body fat percentage, in addition to tracking variations in fat deposition and distribution (Carter et al. 2012). Changes in regional fat deposition in the absence of BW change are primarily influenced by season and exercise, as well as other factors (Dugdale et al. 2010, 2011, Gentry et al. 2004). Although less commonly used and very expensive, TBW is a method that uses deuterium oxide (D<sub>2</sub>O) infusion to calculate total fat versus fat-free mass in the determination of the percentage body fat in live equines (Dugdale et al. 2011, 2012). The deuterium isotope equilibrates into all the body water spaces, excluding lipid tissue. Total body water is calculated according to the amount of isotope administered and its final concentration in blood plasma. Factors that influence

TBW include hydration status and gut fill. Lastly, BIA works through electrical impulses measuring the frequency between electrodes, which is affected by the resistance through tissues. Low frequency electrical current occur in lipid tissue. Although BIA is not commonly used in horses, one study has attempted to use BIA to measure body fat composition in horses and compared these results with TBW and D<sub>2</sub>O dilution methods. In this example, it was found that the results of BIA agreed with those obtained through TBW and D<sub>2</sub>O dilution (Carter et al. 2012, Van der Aa Kuhle 2008). Body composition studies, using these methods, have shown that body fat percentage ranges from 1.12% in thin, athletic horses to 31.3% in obese mixed-breed mares.

#### *1.1 b. Body Condition Scoring*

The scoring of body condition originally developed in production animals as an indicator of “flesh” (lean and adipose tissue). This indicator has now become the most common method of adiposity assessment, making the Henneke body condition scoring system the most widely excepted method for determining body fattness in the US (Henneke et al. 1983). This body condition scoring system, involves the subjective measure of body fat accumulation determined via palpation and observation of subcutaneous body fat along the neck, withers, loin, tailhead, shoulder, and ribs. Each area is scored from 1 to 9, using a detailed description of fat covering, with 1 representing a horse that is emaciated and 9 representing a horse that is grossly obese. The Henneke body condition scoring system was originally formulated for use in Quarter Horses, but has application in all types of equids, including ponies (Freestone et al. 1992), draft breeds (Potter et al. 1987), Thoroughbreds (Suagee et al. 2008), and Warmbloods (Kienzle and Schramme 2004). Kohnke (1992) developed a modification

to the body condition scoring system to increase accuracy by scoring each region separately and averaging the regions to obtain a cumulative body condition score from 1 to 9 (Carter et al. 2012, Kohnke 1992). The limitations to body condition scoring include errors arising from horse factors, such as differences in gender, age, breed, and conformation. Furthermore, this scoring system is a subjective measure, which leaves room for observer interpretation. Despite these limitations, the body condition scoring system is an applicable tool for evaluating fatness of both horses and ponies, and has provided a useful body condition management tool for owners and barn managers (Becvarova 2012, Frank 2010, Suagee et al. 2008).

#### *1.1 c. Relationship between BCS and Body Fat Percentage*

Although body condition scoring is assigned based on subjective interpretations, Laflamme (1997) and Mawby (2004) found that the 9-point BCS system has excellent test-retest reliability in dogs in relation to body fat percentage ( $R^2 = 0.92$ ; in relation with DEXA scan) (Laflamme 1997, Mawby et al. 2004). Dugdale and colleagues (2010) found that a reduction in BW was associated with ultrasonic fat depth ( $r = 0.98$ ). In Welsh pony mares, total somatic soft tissue increased linearly ( $R^2 = 1.00$ ), whereas body white adipose tissue (WAT) increased exponentially with increasing BCS (Dugdale et al. 2011). Thus, higher body fat percentages (from 1% to 26% of BW) are associated with increasing BCS (Carroll and Huntingdon 1998, Dugdale et al. 2011, Laflamme 1997); however, the relationship between BCS and total body WAT suggests that the BCS system loses sensitivity for obese animals. This theory was confirmed by Dugdale and colleagues (2012) who determined that the relationship between BCS and body fat (estimated according to the D<sub>2</sub>O dilution method) in mostly overweight horses was

logarithmic and declines in accuracy for BCSs greater than 7. For a mixed group of horses and ponies (the majority of which were overweight), results showed that the correlation between body fat and BCS was not linear, especially at higher BCSs.

For horses within the 6 to 9 BCS range, estimating body fat from BCS is harder to accurately predict. The graphical representation of the relationship between body condition score and body fat percentage showed less power in the test as BCS increased. However, when using a binary test for 'fatness', horses with an estimated body fat percentage of >20% were those in the 7/9 BCS range, with about 80% test predictability (Matinson et al. 2014). Thus, obesity in horses is classified as a BCS of greater than or equal to 7/9, with a body fat percentage above 20% to 25%. Likewise, it is recognized that some breeds retain more body fat than others (e.g., Andalusians and Paso Finos), and since the BCS system declines in sensitivity as BCS increases, the measurement of body fat by ultrasound in addition to BCS has been employed to establish a more accurate body condition assessment (Martin-Grimenez et al. 2016). Results suggested that not all areas correlated well with BCS and were affected by age and gender; however, subcutaneous fat thickness in the tailhead and rump regions were closely associated with BCS ( $p < 0.001$ ) (Freestone et al. 1992, Martin-Gimenez et al. 2016, Quinn et al. 2006).

#### *1.1 d. Optimal Condition*

Optimal body condition depends on the work-related discipline and physiological status of the horse. Body condition is a known factor affecting athletic performance. Underconditioning may affect energy reserves and energy-containing components in muscle needed to maintain athletic performance (NRC 2007, Scott et al.

1992). Likewise, lean to moderate conditioned horses had higher endurance race completion rates, whereas no horses below a BCS of 3 completed the race (Garlinghouse and Burrill 1998, NRC 2007). Although there is some contradicting evidence (Carter et al. 2012, Fitzgerald et al. 2003, Vick et al. 2006), some studies report advantages on reproductive performance in broodmares when a BCS of 7 to 9 was maintained (Cavinder et al. 2005, Gentry et al. 2002, Henneke et al. 1983, Kubiak et al. 1987). Despite the potential advantages in broodmares, Hoffman et al. (2003) found that horses with a BCS  $\geq 7$  exhibited decreased insulin sensitivity, and ponies with a BCS  $\geq 7$  had an increased risk of laminitis (Carter et al. 2009, 2012, Treiber et al. 2006). Furthermore, increased adiposity is associated with hyperinsulinemia, hyperleptinemia, dyslipidemia, insulin resistance, and increased pro-inflammatory cytokine concentrations (Buff et al. 2002, Carter et al. 2012, Frank et al. 2006, Geor et al. 2008, Vick et al. 2007); thus optimal body condition for most horses and ponies typically range from 4 to 5 and 5 to 6 for broodmares (Carter et al. 2012, NRC 2007).

#### *1.1 e. Morphometric measurements*

Morphometric measurements can be used to evaluate and monitor overall changes in body condition and fat deposition, and some measurements can be used to calculate body weight in horses (Carter 2008, Carroll and Huntingdon 1988, Webb and Weaver 1979). Commonly assessed areas of the horse include, neck circumference, belly circumference, withers height, girth circumference, and body length. Carroll and Huntingdon (1988) developed a BW equation using girth circumference and body length that can estimate actual BW in adult, light breed horses ( $\text{girth}^2 \times \text{length} / 11880$ ). Body length is measured from the point of the shoulder to the point of the buttocks and

girth circumference is measured from just behind the highest point of the withers.

Owen and colleagues (2008) revised the denominator for ponies (10,787). For larger Draft and Warmblood horses, Catalano et al. (2016) devised a model equation for ideal BW that was fit using horses with a body condition score of 5, a body length (BL) measurement that was measured from the point of the shoulder to the end of the body (not wrapped around the buttocks), and wither's height. Bodyweight (kg) was estimated as  $\text{girth (cm)} \times 1.528 \times \text{BL straight (cm)} \times 0.574 \times \text{height (cm)} \times 0.246 \times \text{neck (cm)} \times 0.261 / [1,181 \text{ (draft) or } 1,209 \text{ (warmblood)}]$ . Martinson and colleagues (2014) devised an equation to better estimate body weight by including parameters, such as breed type, height, and neck circumference. In assessing overall adiposity in horses and ponies, BCS may have the strongest association with heart girth ( $R^2 = 0.91$ ), belly girth ( $R^2 = 0.82$ ), and mid-neck circumference ( $R^2 = 0.75$ ) when adjusted for wither's height (Carter et al. 2012, Dugdale et al. 2011). Carter and colleagues (2008) developed a ratio assessment to predict if a horse or pony is overconditioned or obese. In this equation, a girth to height (G: H) ratio of  $\geq 1.26 \pm 0.01$  identifies a horse as overweight ( $\text{BCS} \geq 7$ ;  $1.33 \pm 0.01$  for ponies), and a  $\text{G: H} \geq 1.29 \pm 0.01$  identifies a horse as obese ( $\text{BCS} \geq 8$ ;  $1.38 \pm 0.01$  for ponies) ( $R^2 = 0.64$ ,  $p < 0.001$ ;  $R^2 = 0.83$ ,  $p < 0.001$ ) (Carter et al. 2008, 2012, Dugdale et al. 2011). Dugdale and colleagues (2010) noted that body mass reduction was associated with heart girth ( $r = 0.98$ ), belly girth ( $r = 0.88$ ), and rump width ( $r = 0.95$ ). Martinson and coworkers (2014) used a BW scoring system to assess the likelihood that an individual is overweight or underweight on a basis of morphometric measurements using a logistic regression fit to  $\text{BCS} (0.021 \times (\text{estimated ideal BW} - \text{estimated BW}) + 14.25 \times (\text{neck circumference-to-height ratio}) + 0, 0.497, \text{ or } 0.045 \text{ for}$

Arabians, ponies, and stock horses, respectively). Overweight ( $\geq 7/9$ ) and underweight ( $\leq 4/9$ ) animals resided at the 48<sup>th</sup> and 83<sup>rd</sup> percentile cutoffs, respectively (Martinson et al. 2014).

#### *1.1 f. Regional Fat Deposition*

In some horses, fat is not evenly distributed. Regions of subcutaneous fat accumulate in certain areas including the neck, behind the elbow, over the back and rib, base of the tailhead, and/or around the sheath in males (or udders in females). Even horses with a lean to moderate body condition may have a prominent cresty neck. The mid-neck circumference measurement evaluates neck thickness and dorsal fat deposition above the nuchal ligament at the widest point of the neck. The measurement is taken with a measuring tape half way between the poll and the withers when the neck is in natural carriage (Carter et al. 2012, Frank 2010). Cresty neck scoring (CNS) is a valuable measure of regional adiposity in the neck, which is of particular interest since it has been associated with indices of metabolic irregularity and Equine Metabolic Syndrome (Carter et al. 2009, Frank et al. 2006, Johnson et al. 2002, King and Mansmann 2004). A cresty neck score ranges from 0 to 5, and is often used in conjunction with the body condition scoring system (Fig. 1). Ponies with a  $CNS \geq 3$  had a greater risk of hyperisulinemia and pasture-associated laminitis (Carter et al. 2007, 2008). A neck crest to withers height ratio exceeding 0.71 was associated with a higher likelihood of laminitis, according to Johnson (2010) and King and Mansmann (2004). Carter and colleagues (2008) found that crest height was strongly associated with CNS and higher blood concentrations of insulin, glucose, triglyceride, and leptin.

## 1.2 Overweight Horse Population Statistics

### *1.2 a. Modern Husbandry*

Horses are continual grazers and hindgut fermenters. They require continual digestion of forage to maintain intestinal health. Modern nutritional and management practices used in domesticated horses vary greatly from what a wild horse would encounter (Dugdale et al. 2010, Scheibe et al. 2003). Wild horses experience substantially more exercise than most domesticated horses because of their need to roam in search of food (Argo 2010). Fresh forage available to horses in the wild is less energy dense and higher in cellulose than the pasture maintained for domesticated horses. In addition, horses in captivity are provided with supplemental hay and concentrate at all times of the year, blanketing in winter, routinely stalled, and other provisions that conserve energy and prevent weight loss. This prevention of weight loss during winter combined with seasonal cycles of weight gain during spring and summer months in domestic horses promotes weight gain (Argo 2010, Frank et al. 2010, Johnson et al. 2010, Thatcher et al. 2012).

Thatcher and colleagues (2012) reported that 54% of the horses had continuous access to pasture and more than half of which consumed supplemental hay and/or concentrates. In addition, more than 50% of horses were not receiving any exercise. This was similar to results from an American Horse Council (2005) study that reported 42% of horses are used for recreation purposes, with an exercise classification of idle or light work. In a sample of pasture-fed equines, Giles and coworkers (2014) reported that 71% of ponies and 61% of horses were occasionally exercised, which was similar to results by Robin and coworkers (2015) that reported 62% of horses were primarily

used for pleasure riding. In addition Robin and colleagues (2015) reported that 96% of horses (located in Great Britain) had access to pasture, with 86% receiving supplemental grain that increased in prevalence to 91% over the winter. The risk of obesity was greater in non-competition horses, with pleasure horses being more than twice as likely to be obese and idle horses being nearly 3 times more likely to be obese. The combination of unrestricted, high quality, lush pasture, supplemental hay/concentrate, and little to no exercise sets the prime scenario for obesity, with an especially high risk group being middle-aged horses and ponies (Thatcher et al. 2012, Robin et al. 2015).

#### *1.2 b. Incidence of Obesity*

It is generally accepted that obesity is defined as a BCS  $\geq 7$ , and was initially introduced by Hoffman and colleagues in 2003. Obesity is a growing problem in the horse industry, particularly because of its prevalence and the consequence of disease. Around 50% of horses have a BCS  $\geq 6$  in the US and UK, with a smaller proportion considered obese. In a recent study by Thatcher and colleagues (2012), 47% of 300 mature horses were moderately conditioned (BCS 5), 51% were overconditioned/obese (BCS  $\geq 7$ ), and 19% were obese (BCS  $\geq 8$ ). In 2010, Pratt-Phillips and colleagues found that out of 366 client-owned horses, 48% were overconditioned (BCS  $\geq 6$ ) and 20% were obese (BCS  $> 7$ ). At a national affiliated show, Harker and colleagues (2011) found that 62% were considered overconditioned or obese (BCS  $> 5$ ), with 21% of those classified as obese (BCS  $\geq 7$ ). In a study conducted in south-west Scotland found that 45% of the horses were obese (Wyse 2008). These studies demonstrate the incidence of obesity in

a small subset of the equine population. However, it is undeniable that the incidence of overconditioning in the equine population is prevalent and on the rise.

### *1.2 c. Owner Perception of Fatness*

The nutritional management of animals is greatly influenced by the owner's knowledge and perception of health and condition. In the study by Wyse and colleagues (2008), horse owners were asked to assign their horse a binary score of obese or non-obese. Approximately 50% of horse owners scored their horse incorrectly based on evaluation of that same horse by a professional using the body condition scoring system (Wyse et al. 2008). Similarly, the owner's perception of fatness has been assessed in canines. Rohlf and colleagues (2010) assigned body condition scores to 182 client-owned dogs and compared this with owner's assigned BCS. Results from the BCS assessment showed that approximately 55% of the dogs were defined as overweight or obese by the professionals, however, nearly half of owners (40%) of overweight or obese dogs underestimated the body condition score even after they were provided with a body condition score of their animal by the professionals. Both studies clearly show that owners are oftentimes incapable of recognizing overweight and obese pets or have a problem admitting their animal is overweight (Rohlf et al. 2010).

## *1.3 Risk Factors of Obesity*

### *1.3 a. Consequences of Obesity*

Obese horses are prone to a number of health concerns. Young, overconditioned horses can develop osteochondrosis, a developmental orthopedic disorder, when fed large rations of sweet feeds (Johnson et al. 2012). Overconditioned horses may also

suffer from heat stress (Cymbaluk and Christison 1990, Quinn 2007, Webb et al. 1989). Webb and coworkers (1989) found that horses with a BCS of 7.5 had a harder time dissipating heat in hot weather (temperature 32 °C and 44% humidity) than horses with a BCS of 5. Obese individuals exhibit exercise intolerance because of the high cardiovascular and musculoskeletal demands of carrying additional weight. The reduction of body fat would result in a lower energy requirement for exercise, improving performance. The percentage of body fat by measuring rump fat thickness was negatively correlated with race performance and slower race times (Kearns et al. 2002), which was similar to results by Lawrence and coworkers (2002). Obese mares often have altered estrus cycles, leading to complications when breeding (Frank et al. 2010, Vick et al. 2007). Perhaps most importantly, obese horses are at higher risk for developing laminitis (Geor 2009).

### *1.3 b. Laminitis*

Laminitis is an extremely painful condition resulting in permanent lameness and death in horses. For this reason, laminitis is considered a major clinical disease. The causes of laminitis has been attributed to inflammation by pro-inflammatory factors, gram (-) sepsis/endotoxicity (from strangulating gastrointestinal disease, septic pleuroneumonia, septic metritis, and euterocolitis), trauma, or the carbohydrate overload model (Carter et al. 2009, Harris et al. 2006, Johnson et al. 2010, Suagee et al. 2011, Treiber et al. 2006). Of the types of laminitis, the endocrine-mediated form of laminitis, stemming from dietary origin, remains relatively unreported because of the low-level symptoms that may occur, but is quite possibly the most common form of laminitis. Endocrine-mediated laminitis is usually monitored by owners and local

veterinarians (most cases can generally avoid long-term hospitalization). Laminitis can also be induced through the experimental administration of supra-physiological levels of intravenous insulin to ponies (Aspin et al. 2007; McGowan et al. 2008). De laet and colleagues (2010) induced laminitis in eight Standardbred horses injected a supra-physiological bolus of insulin. In addition, elevated insulin may be involved in the production of inflammatory cytokines (TNF  $\alpha$  and IL-6) which have been implicated in the mechanism of laminitis (Suagee et al. 2011). The mechanism of laminitis involves a speculative sequence of hormonal, inflammatory, and/or vasculatory events that ultimately lead to the detachment of the sensitive laminae from the insensitive laminae of the hoof, causing downward rotation and sinking of the third phalanx (pedal bone).

In most dietary models (carbohydrate overload model and pasture-associated laminitis), alterations to the hindgut environment increase permeability to the absorption of vasoactive amines, exotoxins, and endotoxins that initiate and inflammatory response, leading to laminitis. The gram-positive lactic acid bacterium, *Streptococcus bovis*, has been isolated from the equine cecum in horses that consumed high quantities of starch. Milonovich and colleagues (2006) found that fecal specimens from affected horses contained the genus, *Streptococcus infantarius ssp. coli* as the predominant organism present in horses prior to the onset of laminitis. The presence of certain bacterial species may decrease luminal pH, which increases intestinal permeability. These alterations in the gut result in ischemic events that lead to the release of vasoactive monoamines from the hindgut that trigger laminitis (Bailey et al. 2004). In addition, an unknown trigger factor may be responsible for activating the circulation of metallic matrix metalloproteinases (MMPs) in the hoof wall that break

down the cellular bond which anchors the hoof wall to the pedal bone (King and Mansmann 2004). The MMP gene encodes an enzyme that breaks down collagen, a major component of basement membranes. MMPs were found to be prevalent in laminitic horses induced by the non-structural carbohydrate oligofructose overload model. Oligofructose is a subcategory of fructan that has been seen to induce lameness and reduced fecal pH. In addition, there is evidence that fructans increase blood glucose and insulin levels. Bailey and colleagues (2008) found that previously laminitic ponies demonstrated a 5.5-fold increase in serum insulin concentration versus a 2-fold increase in non-laminitic ponies when fructan (3g/kg d) was included in their diet for 48 h. Therefore, high non-structural carbohydrates and fructans should be avoided in horses predisposed to hyperinsulinemia and insulin resistance because of the high risk of developing laminitis.

Pasture-associated laminitis (PAL) accounts for approximately 50% of all laminitis cases (USDA National Animal Health Monitoring System, 2000). Seasonal elevations in insulin concentration have been observed in grazing horses because of the increase in the non-structural carbohydrates (NSC) content of pasture. Cool season grasses accumulate NSC during the spring and fall and fluctuate throughout the day. Non-structural carbohydrate concentrations rise throughout daylight hours, peaking in the late afternoon and falling in the early morning. Fructan accumulation in cool season grasses may be detrimental to the horse and can lead to the development of laminitis (Bailey et al. 2008). Fructans are the dominant NSC in cool season grasses (Underander 2013). Fructans cannot be broken down by enzymes in the horse's small intestine and travel to the hindgut where they are rapidly fermented by resident microbes. The

excess intake of simple sugars from plant material greatly increases a horse's risk for developing laminitis during a single meal or by chronic over-eating (King and Mansmann 2004). Adaptation to lush pasture can change bacteria flora, with the proliferation of gram-positive, lactic acid producing microorganisms (Daly et al. 2012, Geor 2009, Julliand et al. 2006, Milinovich et al. 2006, Rafat et al. 2009).

### *1.3 c. Equine Metabolic Syndrome*

Laminitis is commonly associated with endocrine diseases, such as Equine Metabolic Syndrome (EMS) and Cushing's Disease (Burns et al. 2015). Horses with EMS usually present with regional adiposity (CNS>3) and obesity, accompanied by insulin resistance (IR) and/or a history of laminitis (McCue et al. 2015). Breeds most commonly affected by EMS are Morgans, Paso Finos, Andalusians, Quarter Horses, and pony breeds (Bailey et al. 2013, Frank et al. 2010, Johnson et al. 2010). Certain breeds have a known tendency to require fewer calories and exhibit enhanced metabolic efficiency, meaning that they require fewer calories to maintain body weight when compared with unaffected animals. McCue and colleagues (2015) and Robin et al. (2016) propose that genetic factors influence the susceptibility of EMS and laminitis across breeds and within individuals. Ultimately, the summation of genetic and environmental risk factors determines the prevalence of disease risk. In a study of owner-reported disease prevalence, Robin and colleagues (2016) found that breed was a significant risk factor for obesity. Morgans, Quarter Horses, and Paint Horses had significantly higher BCS than Thoroughbreds, which was similar to findings by Thatcher et al. (2012). In addition, ponies with a history of laminitis had a significantly higher BCS than non-laminitic ponies.

### *1.3 d. Obesity and Inflammation*

Although traditionally thought of as a fat storing organ, adipose tissue is responsible for complex endocrine activities. Recent research has shown that the endocannabinoid system and the microbiota brain-gut axis are involved in the regulation of energy balance and the development of obesity and systemic inflammation in humans (Cluny et al. 2012). Adipose tissue plays an important role in insulin insensitivity through the action of adipokines and adiposites. Circulating free fatty acids from adiposities are elevated in many insulin resistant conditions and have been proposed to contribute to diabetes by inhibiting glucose uptake, glycogen synthesis, and glucose oxidation. Animal models have shown that obesity results in the progressive development of adipose tissue dysfunction, including systemic inflammation and the development of IR (explained below). However, the degree of metabolic dysfunction varies from individual to individual, with visceral adiposity more strongly associated with inflammation and IR. In humans, mesenteric and omental adipose tissue may be more pivotal in the development of metabolic disease than subcutaneous adipose tissue because free fatty acids and adipokines released from these visceral sites enter the portal circulation and have a more profound effect on hepatic metabolism and insulin clearance. For horses, this would mean that adipose tissue from the neck crest or abdomen may have higher tissue level concentrations of adipokines.

Burns and colleagues (2015) found that there was a higher mRNA expression of pro-inflammatory cytokines in nuchal ligament adipose tissue of IR horses, compared with tissue samples from other regions. Obesity is now being characterized as a

systemic, low-grade inflammatory condition in which adipose tissue releases adipokines, such as leptin, resistin, adiponectin, visfatin, apelin, in addition to pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF $\alpha$ ) and interleukins 1 and 6 (IL-1 and IL-6) into systemic circulation, leading to endocrine imbalances which may result in hyperinsulinemia and laminitis (Cluney et al. 2012, Frank et al. 2010, Suagee et al. 2011, Treiber et al. 2009, Vick et al. 2007). In humans, there is an inverse correlation between adipose tissue IL-6 content and insulin responsiveness (Bastard et al. 2002). However, it is thought that the presence of systemic inflammatory cytokines in chronically overweight horses may contribute to more global inflammation and that pro-inflammatory cytokines could be more representative of insulin status (Adams et al. 2009, Bailey et al. 2013). However, when attempts have been made to reproduce the inflammatory effects of high carbohydrate feeding, no up regulation of laminar pro-inflammatory cytokine or chemokine gene expression was noted (Burns et al. 2015). In one study, the systemic concentration of IL-1, IL-6, and TNF- $\alpha$  were no different between lean, moderate, and obese horses (Burns et al. 2010, Holbrook et al. 2012). The inflammatory effects of obesity-related IR may be dependent on additional horse-related (metabolic gene expression) and/or environment-related risk factors (Frank et al. 2010, McCue et al. 2015)

### *1.3 e. Insulin Resistance*

Overweight horses are at high risk for developing insulin resistance (IR). The protein hormone, insulin, is released from  $\beta$  cells from the pancreases in response to rising blood glucose. When insulin binds to its receptor in the target tissues, glucose is taken up by the GLUT4 transporter and translocated into the cell for storage or use in

metabolic process for the generation of ATP. Insulin insensitivity is generally defined as the inability of normal insulin concentration to produce an adequate response from the target tissue to transfer glucose into the cell. In addition, insulin insensitivity has been linked to an alteration in glucose metabolism within the cell. Therefore, horses with IR require additional insulin concentration to clear blood glucose (Geor 2010, Hoffman et al. 2003, Johnson et al. 2010, Kronfeld et al. 2005). Insulin resistance is suspected when a resting blood sample (food withheld for >12 h) is greater than 20  $\mu$ U/mL (Frank 2006). Methods for more accurate testing of insulin sensitivity in a research setting include the oral glucose tolerance test (OGTT), intravenous glucose tolerance tests (IVGTT), and the euglycemic hyperinsulinemic clamp technique. Intravenous glucose-insulin tests (CGIT) are commonly used in a veterinary setting to evaluate a horse for insulin resistance. A CGIT test evaluates plasma insulin concentration at timed intervals post administration of glucose and insulin. Additionally, testing a 6 to 10 h fasting serum or plasma blood sample for insulin concentration may provide diagnosis for some horses, but not all cases (Johnson et al. 2012). Oral glucose tolerance tests have been used to determine differences in insulin sensitivity between moderate and obese horses (Freestone et al. 1992). The interplay of insulin and glucose dynamics may be equally important in evaluating insulin resistance. The reference range for blood glucose is between 80 and 115 mg/dL. Fasting hyperinsulinemia is usually accompanied by normoglycemia, which suggests compensated IR for the maintenance of glucose homeostasis (McCue et al. 2015). It is thought that a slight elevation in fasting glucose accompanied by IR may be an important factor in evaluating the severity of IR cases and the risk for laminitis (Johnson et al. 2012). Other hormones may contribute to

the upregulation of insulin, such as glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, which are secreted by the gut. Hyperinsulinemia may alter the secretion or action of incretin hormones (McCue et al. 2015).

### *1.3 f. Nutritional Onset of Insulin Resistance*

Feeding diets high in non-structural carbohydrates that support prolonged hyperinsulinemia are thought to induce IR in susceptible horses and ponies (Bailey et al. 2013). Although obesity is associated with increased insulin insensitivity in horses, optimally conditioned horses are also diagnosed with IR, suggesting that the variation in IR may arise from genetic predisposition, age, environmental factors, and activity (Geor 2010, Hoffman et al. 2003, Johnson et al. 2010, McCue et al. 2015, Pratt et al. 2006). Insulin insensitivity in optimally conditioned horses seems to coincide with diets high in NCS and starch. Hoffman and colleagues (2003) found that insulin sensitivity was lower in horses fed pasture and a high NCS supplement, compared to horses consuming pasture and a fat and fiber supplement. In a study by Pratt and colleagues (2006), horses were fed either a sweet feed rich in sugar (S-fed; NSC=53%), a pelleted feed with a high fat ratio (F-fed; NSC=9%) or forage cubes (control diet; NSC=10%). Glucose concentration was unchanged by diet; however, AUC insulin was lower ( $p<0.05$ ) in F-fed horses than the S-fed horses after 6 weeks. The authors concluded that for horses in the S-fed group, a reduction of 30% in the rate of insulin-mediated glucose disposal may result in insulin insensitivity in idle horses fed a diet rich in NSC (Pratt et al. 2006). During a different study, Pratt-Phillips et al. (2014) found that baseline insulin: glucose ratio was higher ( $p<0.001$ ) in horse fed a high NCS diet (compared to horses fed a low NCS diet), concluding that the NCS content of the diet

impacts baseline insulin, the insulin: glucose ratio, and postprandial insulin concentration, with less of an impact of meal size. Similar results were achieved in a study where fourteen adult Standardbred horses were used in a three phase study design: baseline, diet, and diet x exercise. Results from the study showed a decrease in insulin sensitivity in horses fed the high sugar diet (S-diet) versus the high fat diet (F-diet). When exercise was later introduced to both treatment groups, the effects of the S-diet on insulin insensitivity was reversed. For that reason, light to moderate physical activity may be important in reversing or improving the effects of insulin insensitivity in horses (Steward-Hunt et al. 2010). Based on the results of these studies, it is recommended that, diets of fat and fiber are preferable to those high in sugar.

There is considerable variability in the individual response to high glycemic feeds. Ponies tend to be more insulin resistant than horses (Jeffcott et al. 1986, Rijnen and van der Kolk 2003 De laet et al. 2010). Peak insulin response to an oral glucose challenge was highest in ponies and lowest in Standardbreds (Bailey and Bamford 2013, Bamford et al. 2014). Previously laminitic ponies experienced highest peak glucose and insulin concentration after glucose feeding, and area under the curve (AUC) insulin was highest in previously laminitic ponies with both glucose and fructose feeding (Borer et al. 2012). These finding suggests that there may be breed sensitivities to dietary NCS that may affect insulin sensitivity.

### *1.3 g. Obesity and Insulin-Related Statistics*

Insulin concentration positively correlates with increasing BCS ( $p < 0.001$ ) (Carter et al. 2009, Frank et al. 2010, Pratt-Phillips et. al. 2010). In addition, regional adiposity, particularly the enlargement of the neck crest, is positively correlated with

insulin concentration (Carter 2008). Horses and ponies with a cresty neck score of  $\geq 3$  are more likely to be hyperinsulinemic than those with a moderate neck crest score of  $< 3$ . In a study of 34 light breed horses, 21% with a moderate neck (CNS  $< 3$ ) were hyperinsulinemic (insulin  $\geq 30$  mU/L), whereas 40% of horses with a cresty neck (CNS  $\geq 3$ ) were hyperinsulinemic. In an evaluation of 75 mixed breed ponies, only 6% with a moderate neck were hyperinsulinemic, compared to 56% with cresty necks (Carter et al. 2009). Therefore, the measurement of cresty neck is implicated with hyperinsulinemia for equines.

After the onset of weight gain, insulin sensitivity decreased by 71% and 80%, respectively, in studies by Carter and colleagues (2009) and Hoffman and colleagues (2003), respectively. Carter and colleagues (2009) reported that 19% of moderately conditioned horses ( $4 < \text{BCS} < 7$ ) were hyperinsulinemic compared to 43% of the overweight and obese horses ( $\text{BCS} > 7$ ). Likewise, ten percent of moderately conditioned ponies were hyperinsulinemic versus 45% of the overweight and 63% of the obese ponies. Thus, it appears that obesity may predispose a horse to hyperinsulinemia, but not all obese horses are equally affected, meaning there other factors affecting the development of IR.

#### 1.4 Energy Requirements

Energy systems have been developed to quantify the energy in feed that is utilized by the animal (NRC 2007). The gross energy of feed is the amount of heat from combustion that is produced in a pressurized chamber called a bomb calorimeter (NRC 2007). Digestible energy (DE) is calculated by subtracting the gross energy in the feces

from the gross energy intake. The DE value of the feed varies between species and individuals. Digestible energy minus the energy from gas and urine (approximately 11%) equals metabolizable energy. Metabolizable energy can be transformed into recovered energy or to heat energy. According to Vermorel and colleagues (1997), metabolizable energy ranges from 84.6% to 90.5% of digestible energy.

The National Research Council (2007) has developed energy requirements in horses based on the physiological status of the animal and the amount of forced physical activity the animal is undergoing. The most basic requirement is referred to as 'maintenance,' which applies to adult animals that are not pregnant, lactating, growing, or performing work. The maintenance energy requirement pertains to the energy required for a horse to maintain zero energy balance, which is the energy that is required to maintain cellular processes, thermoregulation, and normal daily activity. Maintenance energy requirements have been estimated in mature horses using indirect calorimetry, metabolic balance trials, and feeding trials in a confined environment. Pagan and Hintz (1986) found that maintenance energy requirements increase linearly with increasing body weight. A large number of studies have estimated metabolizable energy by measuring daily heat production. By utilizing the metabolizable energy to digestible energy efficiency of conversation (approximately 87% according to pooled studies), the DE intake estimated across studies ranged from 25.7 to 35.1 kcal/kg BW (NRC 2007) in a thermoneutral environment. Although the mean DE intake from pool studies averaged 30.3 kcal/kg BW (from horses in confinement), the NRC assumes a value of 33.3 Kcal/kg BW/d to represent mature, idle horses with some voluntary activity and an average temperament. The NRC developed a range of 10% above and

below maintenance requirements to account for differences in horse temperament and voluntary activity: 30.3 kcal/kg BW for reduced energy, sedentary horses (minimum maintenance requirements), and 36.3 kcal/kg BW for alert temperament horses with moderate voluntary activity (elevated maintenance requirement). It is recognized that there may be additional horse to horse variation in maintenance energy requirements based on genetic variation and the myriad of husbandry situations that occur worldwide. Variation in DE intake for zero energy balance exists from horse-related factors, such as age, breed, temperament, body mass, metabolic efficiency, stress, etc. Therefore, the variation in maintenance DE requirements may be greater than the NRC (2007) has predicted.

#### *1.4 a. Digestible Energy in Feed*

The digestible energy in feed is the amount of energy available to the horse for use in maintenance, growth, production, lactation, and performance. Pagan (1998) has developed an equation to estimate the digestible energy (kcal/kg DM) from feed:  $2,118 + 12.18 \times (\% \text{crude protein}) - 9.37 \times (\% \text{acid detergent fiber}) - 3.83 \times (\% \text{hemicellulose}) + 47.18 \times (\% \text{fat}) + 20.35 \times (\% \text{non-structural carbohydrate}) - 26.3 (\% \text{ash})$ ;  $R^2 = 0.88$ , where hemicellulose = acid detergent fiber – neutral detergent fiber and non-structural carbohydrate =  $100 - \% \text{neutral detergent fiber} - \% \text{fat} - \% \text{ash} - \% \text{crude protein}$ . The above equation may overestimate the DE content of high fiber and fat feeds. The DE digestibility of a feed can be altered by feed processing (whole vs. steamed, crimped, rolled or popped), and there is also the possibility that one diet form may affect the digestibility of another diet form when fed together (Jansen et al. 2000, 2002). The

energy digestibility of a feed is most profoundly affected by differences in digestibility between animals (NRC 2007).

#### *1.4 b. Diet-Related Factors*

It is recognized that the energy requirement for horses vary considerably based on the previously mentioned criteria. Digestibility of a diet is related to horse- and diet-related factors. Heat energy would be greater with highly fermentable forage diets than when mixed diets were fed. Jansen and colleagues (2007) found that feeding fat significantly depressed fiber digestibility, thus reducing the amount of energy provided by dietary fiber. Martin-Rossett and Vermorel (1991) did not find that breed or feeding level resulted in an appreciable change in diet digestibility; however, Vermorel and colleagues (1997) found that maintenance energy requirements were lower in ponies than horses. Pagan (1998) found that mixed diets were more digestible than forage diets. However, the fiber in the forage diet was more digestible than the mixed diet. Likewise, Edouard and colleagues (2008) found that dry matter digestibility declined with forage quality and that the effect of the individual played a large role in digestibility and forage intake.

#### *1.4 c. Environmental Effects*

Horses regulate body temperature in response to heat or cold. The thermoneutral zone (TNZ) is the ambient temperature where the animal does not need to adjust its body temperature to maintain thermostability. Temperatures below the lower critical temperate (LCT) initiate metabolic heat production; conversely, temperatures above the upper critical temperate (UCT) initiate evaporative heat loss by sweating. The ambient temperature to which a horse is adjusted to qualifies for the

TNZ. Based on data from grazing horses, acclimation to short-term thermal stress required 10-11 days; thus temperature values of the LCT and UCT are dynamic (Senft and Rittenhouse 1985). When temperatures are cold and wet, the animal's maintenance needs increase to maintain body temperature. In one study, mares were fed 150% of recommended intake during cold, wet weather but still lost weight (Kubiak et al. 1988). Obese horses may fair better in cold environments; one of the benefits of excess body fat is insulation from the cold. Cymbaluk and Christison (1990) found that obese horses required less supplemental feeding over the winter, possibly because of the availability of excess body fat as an energy source. Horses experiencing cold stress require intakes of 2.5% above maintenance for every 1°C below the LCT (Cymbaluk and Christison 1990). The increase in the maintenance requirement for horses exposed to the UCT has not been quantified; however, it has been assumed that obesity contributes to heat stress during hot weather (Cymbaluk and Christianson 1990, Quinn et al. 2008). Webb and colleagues (1989) found that overweight horses had a more difficult time dissipating heat in hot and humid environments, according of higher respiration rates (and heart rates) during exercise. Fibrous feeds often aggravate heat stress because of the heat increment of feed is high in comparison with feeding concentrates or fats (Cymbaluk and Christison 1990). Thus, research pertaining to the exact metabolic quantification of heat or cold-related stressors in pasture-fed horses requires more study.

## 1.5 Controls of Appetite and Feed Intake

Horses are designed to consume small, frequent meals, which reflect the low holding capacity of the stomach and the down-regulation of digestion products in the small intestine (Frape 2004). Appetite is controlled by the synergy of many mechanisms, including digestive products, energy balance, gastrointestinal tract physiology, chemoreceptors, and hormonal control. Long term signals are involved in the maintenance of body weight and adiposity by controlling food intake and energy balance (*i.e.*, leptin, insulin, and ghrelin), whereas short-term signals influence satiety and the size of the meals. Synergistically, this regulatory system aids in the maintenance of energy balance.

### *1.5 a. Hormonal Regulation*

The interaction of satiety hormones is essential in maintaining zero energy balance. Leptin is an important hormone that controls food intake by signaling satiety to the brain through receptors in the hypothalamic and brainstem regions of the central nervous system. Leptin suppresses the release of endocannabinoids, resulting in the activation of  $\beta 3$  adrenergic receptors in brown fat, thereby increasing fatty acid oxidation. Leptin concentration is affected by feeding level; generally declining during fasting and increasing in overfed, obese animals (in concert with insulin concentration). Delavaud and colleagues (2000) found a positive correlation between adiposity and leptin concentration in ewes, which is also seen in horses (Pratt-Phillips et al. 2010). Leptin acts on sites of the CNS to regulate food intake and promote energy expenditure. In human and rodent models, leptin concentration is generally correlated with fat mass; leptin concentration is generally high in obese horses (Pratt-Phillips et al. 2010).

Neuropeptide Y is a neuromodulator expressed in the hypothalamus that stimulates feeding, especially in response to a carbohydrate-rich diet. In rats, long-term exposure to a high carbohydrate diet lead to obesity and the down regulation of NPY, a counter-regulatory model thought to diminish energy intake and limit the progression of obesity. Insulin and leptin regulate the synthesis and release of NPY for the regulation of energy homeostasis by inhibiting NPY-mediated neuronal activities. The experimental administration of NPY to the brain markedly increases food intake in animals, without adaptation, accelerating weight gain (Marsh et al. 1998).

Ghrelin is a neuroenteric peptide that stimulates food intake, adiposity, and growth hormone release in human and rodent models. Neural and humoral signals from the gastrointestinal tract transmit messages of satiety and starvation to the brain via the afferent vagal nerve, systemic circulation, or both. Ghrelin is secreted in response to starvation and acts predominantly on feeding behavior and the release of growth hormones via the gastric vagal afferents. In rodents, the infusion of systemic ghrelin increases food intake and body weight. In addition, ghrelin increases the respiratory quotient (RQ), suggesting a switch from fatty acid oxidation to glycolysis. Plasma ghrelin concentrations in rats increased upon fasting and returned to baseline after re-feeding. In humans, fasting plasma ghrelin concentrations negatively correlates with percentage body fat, fasting insulin, and leptin concentrations. The downregulation of ghrelin is recognized in human obesity and upregulated during negative energy balance situations, *i.e.*, starvation or anorexia, to relay anabolic signals (Nakazato et al. 2001, Shiiya et al. 2002, Wren et al. 2001).

### *1.5 b. Physiological Mechanisms*

Physical sensors of food entering the digestive system are triggered by mechano- and chemo-receptors. These chemical and stretch receptors 'sense' the presence of food during the initial stage of digestion. Chemoreceptors respond to the digestion products, sugar, fatty acid, and amino acid (Havel 2001), and stretch receptors are activated by the entry of food into the stomach and small intestine. These physical and chemical systems play an important role in the short-term regulation of food intake by limiting the size of the meal and meal frequency.

### *1.5 c. Digestive Products*

The products of digestion (i.e., VFAs, fat, protein, and glucose) may contribute to satiety in mammals. Volatile fatty acids (VFA) contribute over 80% of energy requirements in horses, especially when the diet is high in fiber (Vermorel et al. 1997). In fasted ponies, high cecal concentrations of VFAs (*e.g.* propionate) induce an immediate but minimal depression of appetite by extending the intervals between meals and reducing meal size (Ralston et al. 1983). Likewise, the feeding of fat appears to increase the duration of satiety after a meal (Houpt 2006). Additionally, as previously mentioned, the presence of fat in the duodenum triggers the release of CCK from endocrine cells that has been shown to inhibit food intake in murine and human models. Although feeding fat may have an inhibitory effect on intake, fat is a high energy substrate that can significantly increase energy intake, leading to body weight gain and obesity in some animals. In human and animal models, a low circulating glucose concentration will trigger the sensations of hunger. However, in horses, satiety doesn't appear to be affected by changes in blood glucose concentration (Frape 2004).

### *1.5 d. Energy Balance*

While hormones are in place to control appetite, the amount of calories ingested versus those expended may not properly align, producing positive or negative energy balance. The change of season affects forage supply and quality, resulting in cyclical changes in body weight, which could arise from factors such as climate (cold vs. hot weather thermoregulation), feed availability, and feed quality (most notably affecting pasture-fed horses). When body weight was plotted against time, studies have shown that horses may increase in weight throughout the spring (peaking over the summer), followed by progressive weight loss over the autumn and winter (Berger et al. 1999; NRC 2007, Giles et al. 2015). Because domesticated horses are fed supplemental rations over the winter when pasture supply is low, there is considerably less change in BW for domesticated horses in comparison with feral horses.

### *1.6 Weight Management*

There are important welfare concerns for horses that are significantly underweight (BCS<2.5/9) or overweight (BCS>7/9) (Christie et al. 2005, Geor 2008, Kronfeld 1993, Muñoz et al. 2010, Stull 2009, Suagee et al. 2013). Energy balance can be manipulated to produce weight gain for undernourished horses. Several studies have investigated feeding regimens for such horses to recover from starvation (Witham and Stull 1998, Kronfeld 1993). Stull (2003) found that gradually refeeding starved horses' alfalfa hay produced the lowest insulin response and similar weight gain in comparison with a concentrate and oat hay diet, while providing a high quality nutrient profile (NRC 2007).

The relationships among DE intake for BW gain have been described in horses being fed towards obesity (Carter et al. 2009, Lindåse et al. 2016). Suagee and colleagues (2008) fed 56 kcal/kg BW/d and reported 89 kg of BW gain over an 8-month period. Heusner (1993) found that mature horses required 24 Mcal DE above maintenance per kg of BW gain. Martin-Rosset and Vermorel (1991) found that mature Standardbred geldings required 18 Mcal DE/kg gain. Previous studies have shown that the amount of weight gain in a particular horse is dependent on addition factors, such as the maturity of the horse (Cymbaluk et al. 1989), composition of the diet (Bush et al. 2001, Martin-Rossett and Vermorel 1991), body condition of the horse (Webb et al. 1990), basal metabolism (Stillions and Nelson 1972, Potter et al. 1987), and energy expenditure (Graham-Thiers et al. 1991, Pagan and Hintz 1986). The National Research Council (2007) suggests that 16 to 20 kg of BW gain is equivalent to one unit increase in BCS; although Thoroughbred horses may require 32 kg per unit BCS change (Quinn et al. 2008). Therefore, breed specific difference may significantly affect BCS change or different breeds may carry their weight in different places, affecting body condition scoring.

#### *1.6 a. Weight Loss Statistics*

The goal of managing obese equines centers on 1) controlling calories in and 2) increasing energy expenditure. Weight loss management in horses often includes a reduction in the quantity and quality of the ration and an increase in physical activity through forced exercise (Argo et al. 2012, Dugdale et al. 2010, Geor and Harris 2009, Gill et al. 2016, McGowan et al. 2013, NRC 2007). Weight loss trials have been successfully conducted in an experimental setting, in the absence of pasture. Obese

ponies fed a forage-based diet at 0.75% BW and 67% of maintenance energy requirements for 12 weeks lost  $11.4 \pm 1.9\%$  of BW ( $28.9 \pm 3.5$  kg); however, the changes in BCS was minimal ( $0.3 \pm 0.14$ ) (Dugdale et al. 2010). Van Weyenberg and colleges (2008) successfully induced weight loss in nine obese ponies, by feeding a successive reduction of 70%, 50%, and 35% of maintenance energy requirement over 18 weeks. Ponies lost an average of  $18.2 \pm 1.8\%$  of BW and decreased in BCS by 4. McGowan and coworkers (2013) restricted feed intake to 1.25% of BW of a soaked hay ration fed at 65% of maintenance DE requirements and the ponies decreased in weight by approximately 1.1% per week. Body condition score and serum insulin concentration also decreased (McGowan et al. 2013). Argo and colleagues (2012) restricted intake to 1.25% of BW in DM to determine the rate of weight loss in 12 mature horse and pony mares and geldings. Although there was a wide range of body weight lost between individual horses (from 2.41% – 8.28% in week 1), weight loss was relatively linear thereafter (0.44%/wk). Over the last four weeks, half of the animals gained weight; thus energy intake was further restricted in these animals. Circumferential measurements of heart girth and belly circumference decreased linearly overall (Argo et al. 2012).

Differences in metabolism may contribute to the between-horses variability in weight loss seen in previous studies (Argo et al. 2012, Dugdale et al. 2010, Ungru et al. 2012). Individual variation in the rate of weight loss in response to dietary restriction is recognized in human and murine subjects (Bouchard and Tremblay 1997, Rikke et al. 2006). It appears that genetic variation plays a significant role in obesity predisposition. Argo and colleagues coined the terms, weight loss sensitive (WLS) and weight loss

resistant (WLR) to account for difference in metabolic predispositions towards weight reduction. In a survey study, a greater proportion of ponies were described as “good doers” compared with horses (63% vs. 35%; Robin et al. 2016).

Because of the link between obesity, hyperinsulinemia and laminitis, ration formulation for obese horses should be low in sugar and high in fiber. The NSC content for the total diet should be between 10 to 12% of dry matter intake for metabolic soundness in horses (Becvarova and Pleasant 2012, Frank et al. 2010, Johnson et al. 2012, McGowan et al. 2013). Pasture access should be eliminated in high risk, hyperinsulinemic horses or horses with a history of laminitis or hoof sensitivity (King and Mansmann 2004). However, pasture access may be gradually introduced in some horses once body weight and insulin sensitivity has improved. Johnson and colleagues (2002) conclude that the ideal diet for weight loss should consist of a hay-based diet fed at 1.25% to 1.50% of current body weight. Forage should be fed at no less than 1% of the diet, and vitamin/mineral supplementation may be necessary based on a complete diet analysis. Rations should be weighed before feeding to ensure the proper requirement level is being met. Weight loss should be monitored using a scale, weight tape, and/or morphometric measurements. Dietary restriction should be done gradually over several months, with a goal weight loss of approximately 1% of BW per week. Because the horse will be consuming less food, the initial reduction in weight (during the first week) may be greater than 1% of BW because of gut fill.

#### *1.6 b. Risks Associated with Dietary Restriction*

Dietary restriction in obese horses and ponies isn't without risk. Ponies are at especially high risk for hyperlipaemia (anorexia-triggered excessive release of

triglycerides into the bloodstream) if dietary restriction is too stringent. Hyperlipaemia is characterized by abnormally elevated levels of plasma and serum lipids, including elevated triglycerides, free fatty acids, and very low density lipoproteins, which can be diagnosed through increased serum triglycerides. Overall, modest dietary restriction of 1.25 to 1.59% of BW in dry matter from a forage-based diet is generally recommended, with 1% only used for weight loss resistant horses or ponies after 1.25% BM in DM has failed to produce the desired results (Argo et al. 2012). Reducing forage intake may cause gastrointestinal complications or destructive behaviors in horses. Horses are known to increase wood-chewing during confinement (Krzak et al. 1991, Waters et al. 2002; Cooper and McGreevy 2007) or when limit-fed (Hothersal et al. 2009). Wood-chewing infrequently causes small intestinal obstructing due to the ingestion of wood splinters (Green and Tong 1988, NRC 2007). A study by Curtis and colleagues (2011) found that obese ponies consumed a significant (>1 kg FW/d) amount of wood shavings when undergoing dietary restriction of 1.25% of BW. The ingestion of large quantities of straw bedding in horses has been identified as a risk factor for the occurrence of impaction colic (Reeves et al. 1996). Additional risk factors of feeding infrequent large meals include altered hindgut fluid balance because of the changes in osmolarity that result from accelerated fermentation; both potentially contributing to an increased risk of colic. However, Siciliano and colleagues (2012) found that restricted grazing horses exhibited similar hindgut pH and fluid balance as those grazing for 24 h/d. Although highly restrictive diet have known risk factors, multiple studies suggest that modest changes in body weight are safe and can result in metabolic soundness (Argo et al.

2012, Dugdale et al 2010, Geor 2010, McGowan et al. 2013, Treiber et al. 2006, Van Weyenberg et al. 2007).

#### *1.6 c. Effect of Weight Change on Insulin/Glucose Dynamics*

Blood glucose is maintained between narrow limits, generally between 4.5 to 5.5 mmol/L for most mammals. Blood glucose concentration is primarily regulated by the anabolic hormone insulin and the catabolic hormone glucagon. Blood glucose homeostasis is regulated by glucose-stimulated insulin secretion, which operates by preferentially removing glucose from the blood and storing it in metabolically active tissue. Plasma insulin concentration changes dramatically in response to slight changes in glucose concentration.

Body weight reduction has resulted in an improvement in insulin sensitivity. Van Weyenberg et al. (2008) found that insulin concentration decreased significantly with mild to moderate weight loss; however, baseline glucose remained relatively unchanged. Freestone and colleagues (1992) found that 5% weight reduction on an exercising program improved insulin sensitivity in horses. Dugdale and colleagues (2010) reported that approximately 6% BW loss over 6 weeks resulted in the reversal of hyperinsulineamia. Likewise, results from Argo and colleagues (2012) suggest that insulin sensitivity was improved in 75% of the animals, with no effect on baseline glucose concentration. On the other hand, Quinn and coworkers (2008) and Lindase and colleagues (2016) did not find a difference in glucose or insulin dynamics when BCS increased from moderate to obese in mature Thoroughbred gelding and Standardbred geldings that were fed for BW gain.

#### *1.6 d. Human and Animal Weight Loss and Compliance*

In any weight loss study, compliance to the dietary strategy is an important factor. While there are limited reported studies of compliance in horses (Gill et al. 2016), there is a good compilation of studies in humans and dogs. In a human weight loss trial for overweight and obese adults with cardiovascular disease, 69% of phase 1 participants lost a mean of 5.8 kg (BMI -2.0 (1.5) kg/m<sup>2</sup>) and a mean percentage decrease in initial weight of 6.0% over 20 weeks. Attendance to the weekly meetings declined over time, but remained at 73% of participants (Hollis et al. 2008). Greater weight loss was associated with more frequent attendance to group sessions, the number of food records kept over the trial period, and the amount of exercise. Even modest weight loss of at least 4 kg has been associated with health benefits such as prevention and/or treatment of hypertension, diabetes, and lipid management. For Stevens and colleagues (2001), mean weight loss of 2.0 kg led to 20% reduction in risk for hypertension. Staessen and colleagues (1988) reported additional benefits in systolic blood pressure decreasing 1.0 to 2.4 mmHg per kg after weight loss.

Overall, good compliance with weight loss is generally associated with knowledge about obesity and obesity-related illnesses (Rohlf et al. 2010). Kienzle and colleagues (1998) found that owners of obese dogs were more likely to believe exercise and balanced nutrition were less important than owners of normal weight dogs. Additionally, Carciofi (2005) found that barriers, such as lack of time for exercise, and the cost associated with veterinary treatment were the primary reasons preventing compliance with weight loss treatments for owners of overweight canine. Owners who intended to exercise their dogs were more likely to feed them properly. The authors

found that owners were likely to perceive their dog as larger than they actually were. For example, owners of small dogs characterized them as medium dogs, and so forth. This could pose a problem in feeding the proper amount, especially for overweight dogs. Studies by Yaissle and colleagues (2004) and Bissot and coworkers (2010) found that owners were more willing to follow dietary plans once they noticed an increase in their animal's activity level and a decrease in BCS. To improve compliance, weight loss studies in dogs and humans have reported providing educational materials, food and medical care at no or low-cost, and weight checks to increase compliance. Therefore, veterinary and nutritional professionals need to promote the importance of feeding a weight-appropriate, nutritionally-balanced diet and increase perceived behavioral control by providing information and encouragement to owners. Client-mediated weight loss programs can be successful (given motivation by the owner); however, the rate of weight loss is generally lower than in an experimental setting (German et al. 2007).

## 1.7 Role of Pasture in Equine Management

### *1.7 a. Nutritional value*

The nutritive value of pasture is a function of its nutrient content, digestion, and utilization by the animal. Factors affecting the nutritive value of pasture include plant maturity, plant species, and season. Pastures can supply the nutrient requirements of horses if they are stocked appropriately. An abundance of boarding facilities do not properly utilize their pastures because too many horses occupy a small acreage establishment. Pasture can provide year-round forage for horses if managed properly,

where the quality and quantity of the forage yield and the size, workload, and physiological status of the horses are taken into account. There are many benefits to grazing from an environmental and equine perspective (Bott et al. 2013). A pasture containing greater than 70% vegetation can yield approximately 5 t DM/ha in grass, which can be collected to provide annual forage DM for an average horse requiring 10 kg DM/d (NRC 2007). Hunt (1995) and Elphinstone (1981) concluded that 1-ha of grass pasture would sufficiently supply enough herbage mass for most feeding classes, with the exception of horses in heavy/intense work or in extreme climates.

Forages are mainly composed of structural carbohydrates and lignin. Additional concentrations of non-structural carbohydrates (NSC) such as sugar, glucose, sucrose, and storage carbohydrate, such as starch and fructan, are contained within the plant. Fresh forages play an essential role in the maintenance of normal microbial function of the hindgut, which is in turn needed for maximal fiber fermentation and VFA production. Fresh forage intake may be a factor of plant maturity, NSC content, herbage quality and sward height characteristics. Horses are known to discriminate between different grass types (cool and warm season species) and legumes vs. grass varieties in a mixed pasture, based on additional factors, such as NSC content and maturity. Horses select forage based on plant maturity, preferring immature forage to mature forage containing higher lignin content (Fleurance et al. 2001, NRC 2007). Horses have a preference for grazing swards of 4 to 5 cm in height over roughs (taller, unmanaged plants) (Fleurance et al. 2001, NRC 2007). This preference result in 'spot grazing', where some areas are grazed more completely (lawns) and other areas remain tall and

unkept (roughs), leading to incomplete utilization of pasture forage. Proper pasture and grazing management can help reduce these problems.

#### *1.7 b. Energy*

The DE of the plant can be estimated using the crude protein (CP), acid-detergent fiber (ADF), hemicelluloses, fat, and non-structures carbohydrate, and ash concentrations. The ADF, ash and hemicelluloses percentages of the plant increase with increasing maturity, which lowers the DE. Young plants are usually higher in fat, CP, and NSC, which increase the DE. The fiber components of the forage (NDF and ADF) correspond to the amount of voluntary intake and energy provided by the plant, respectively. Cyclical cycles of growth as a factor of season can affect the ADF and NDF content of the plant, and the NDF level is negatively associated with dry matter intake (DMI) in horses. The NSC concentration of the plant varies by day, as well as at hourly intervals. All considered, the DE content of pasture is extremely variable and must be determined routinely via compositional analysis (NRC 2007).

Depending on location, the feeding value of pasture can exceed the nutrient value for some feeding classes, leading to obesity (maintenance-idle, late gestation, light work) (Bott et al. 2013, NRC 2007). An average grass pasture may have between 2.0 and 2.7 Mcal/kg digestible energy. However, from 2001 to 2011, grass analyzed from samples across the nation yielded from 2.16 to 3.00 Mcal DE/kg DM. A summary of energy values from pastures in New Zealand showed that the energy content of 2.4 to 2.9 Mcal DE/kg DM exceeded the maintenance energy requirement provided by the NRC (2007) of 1.90 Mcal/kg DM for a 500 kg horse (Hoskin and Gee 2004). Gallagher and McMeniman (1988) and Martin (1993), found that the digestible energy content of

lush spring/summer pastures were 2.3 to 2.82 Mcal DE/kg DM. Over the winter months, the DE content declined between 1.8 and 2.5 Mcal DE/kg DM. Surprisingly, estimates from these studies, irrespective of season, supply well above NRC (2007) maintenance DE requirements (1.67 Mcal DE/kg DM) for a 500 kg horse, if dry matter supply is not limiting. Siciliano (2012) and Becvarova and Pleasant (2012) estimate that a horse consuming an average quality pasture (2.0 to 2.25 Mcal DE/kg DM) at 2.5% BW would provide an excess 160% of digestible energy maintenance requirements. Therefore, horses kept on lush pasture can achieve and maintain an obese body condition.

#### *1.7 c. Pasture Management*

Based on previous studies, horses will graze between 10 to 17 h/d (Crowell-Davis et al. 1985, Fleurance et al. 2001, Gallagher and McMenniman 1989, NRC 2007). A majority of horses are managed at pasture. Continuous grazing systems can result in the underutilization of mature forage and overutilization of short vegetation, allowing weeds to proliferate. It is estimated that only 50% to 70% of available herbage mass is utilized during continuous grazing because horses are selective grazers. Grazing systems can be implemented to better utilize fresh pasture, promote uniformity of grazing, and prevent areas of overgrazing. Rotational grazing involves the movement of animals from one area to another as herbage availability declines. This allows time for unused pastures to be rested, fertilized, mowed, and for re-growth to take place before horses are re-introduced. Rotational grazing supports uniform forage selection and limits damage to pasture grass. In addition, rotational grazing allows owners to limit the amount of forage available to the horse to prevent overeating.

#### *1.7 d. Methods for Pasture Intake Determination*

The determinate of dry matter intake in grazing horses is difficult to predict because of practical limitations. Several studies have estimated pasture intake by the difference in herbage mass (Duren et al. 1989), the utilization of fecal markers, such as n-alkanes (Chavez et al. 2014), gravimetric (BW change after accounting for insensible weight loss and excretory output) (Ince et al. 2005), the measurement of fecal output when the organic matter is known or DM digestibility (Grace et al. 2002, NRC 2007), and through the determination of bite size, bite number, and duration of feeding (Duren et al. 1989).

Rayburn and Lozier (2003) devised a method of herbage mass estimation using a falling plate meter device to measure the compressed sward canopy height and herbage density so that herbage availability can be estimated. Multiple measurements are taken systematically at random over the entire grazing area. Calibration of the plate meter to the area is done by harvesting short, medium, and tall compressed plants to determine the grams of DM at varying sward heights (Rayburn and Rayburn 1998). Using this method, the difference in the initial and residual herbage mass is used to calculate the removal rate (intake rate) of the horses. Duren and colleagues (1989) estimated DMI in Thoroughbred and Quarter Horse yearlings and determined that the yearlings consumed forage at a rate of 0.62 kg DM/h (2.5% of BW/d when assuming 15 h of grazing). In another study, Duren and colleagues (1987) found that yearlings consumed forage at 3.2 kg DM or 1.07 kg DM/h (when gazing for 3-h sessions). Using a similar technique, Cantillon (1986) determined that mature horses consumed pasture at a rate of 1.5 kg DM/h. Both of these studies present higher intake rates than the NRC

(2007). However, this methodology has the potential of overestimation because it does not account for pasture regrowth. Grace and colleagues (2002) estimated mean DMI and digestible energy intake (DEI) according to the equation,  $DMI = \text{daily fecal DM output} / (1 - \text{digestible DM})$ . The DMI of yearlings and lactating Thoroughbred mares were 1.97 kg DM/100kg BW/d and 2.43 kg DM/100 kg BW/d, respectively. The DEI was 22.29 MJ DE/100 kg BW/d and 26.20 MJ DE/100 kg BW/d, respectively.

Digestibility markers present practical solutions for measuring intake in pastured horses and allow for the estimation of intake from individual horses. A naturally occurring indicator (internal marker) present in the plant (i.e., lignin, acid insoluble ash) can serve as an indigestibility index when measured in the feces and in the diet. External markers can be administered to an animal at a known quantity and measured in the feces. There is some evidence that the internal indigestibility markers, including n-alkanes (Chavez et al. 2014), chromic oxide (Haeleyn et al. 1966, Holland et al. 1998), and acid insoluble ash (Bergero et al. 2004, Bergo et al. 2009, Cuddeford and Hughes 1990, Miraglia et al. 1999, Sutton et al. 1977, Van Keulen and Young 1977) are effective in predicting dry matter intake in animals. In individual animals, the marker concentration in the diet versus the feces provides an estimate of the diet indigestibility or the proportion of consumed diet that is excreted in feces, calculated as:  $\text{marker concentration in feed} / \text{marker concentration in diet}$ . The dry matter digestibility is then calculated as one minus the indigestibility (Dove 2010). Indigestibility markers are not an exact estimation of DMI, but represent an estimation of grazing intake when other methods are impractical or may alter normal grazing behavior.

Plant alkanes are predominately indigestible, odd-chain hydrocarbons, found in plant cuticle waxes (Dove and Mayes 1996). The surface of waxes contains a mixture of straight-chain hydrocarbons, ranging from 21 to 35 carbons. N-alkanes with odd-number hydrocarbons predominate, and the relative amount of n-alkanes differs depending on the plant species. Most species tend to have primarily C29 to C33 odd-chain n-alkanes. Stevens and colleagues (2002) used the n-alkane marker method to evaluate the dry matter intake and digestibility in horses. They found that the n-alkane method gave good estimates of dry matter intake. For example, the measured intake for fresh ryegrass was  $8.86 \pm 0.23$  kg and the estimated intake, according to the C31:C32 ratio and C32:C33 ratios were  $7.9 \pm 1.9$  kg and  $8.3 \pm 1.4$  kg, respectively. Additionally, the apparent DMD of fresh Ryegrass and Kikuyu grass were 53.5% and 58.6%, respectively, using C31, C33, and C35 n-alkanes, which was within the normal digestibility range for pasture grasses. Chavez and coworkers (2014) used a similar method and found that the C31 estimation of DMI of horses grazing non-toxic, endophyte infected tall fescue pasture was 9.54 kg DM/d to 15.56 kg DM/d, which represented 1.75% to 2.74% of BW in DMI. The measured and estimated dry matter digestibility (using C31 and C33 n-alkanes) of the pastures ranged from 45.2% to 60.9% (Chavez et al. 2014). Menard and colleagues (2002) found that the dry matter digestibility of semi-natural grassland (medium quality) was 61% in May, 57% in June, and 53% in July.

The acid insoluble ash (AIA) content of the plant is the proportion that is not hydrolyzed by 2N or 4N hydrochloric acid and is not subsequently volatilized upon the incineration of this acid insoluble residue. This small amount of indigestible substance (oftentimes referred to as silica) has been completely recovered in ruminant and small

ruminant studies (Shrivastava and Talapatra 1962, Van Keulen and Young 1977), with acceptable results observed in horses (Bergero et al. 2004, Miraglia et al. 1999, Sutton et al. 1997, 2009). There are mixed reviews on the difference in laboratory methods (2N HCl vs. 4N HCl) on the determination of AIA content; however, most recent findings indicate that both the 2N or 4N HCl method produce repeatable digestibility coefficients in horses (Bergero et al. 2009). When compared with total collection, dry matter digestibility coefficients obtained by AIA were statistically similar (Cuddelford and Hughes 1990, Miraglia et al. 1999, Orton et al. 1985). Miraglia and colleagues (1999) fed horses a mixed diet (hay: concentrate; 67:33) for two trials. The dry matter digestibility for trial 1 and trial 2 were 61% and 65% for the AIA method respectively, and 58% and 62% via total collection, respectively. According to Miraglia and colleagues (1999), AIA may slightly overestimate actual dry matter digestibility. Low AIA content in feedstuffs may result in analytical error; therefore, precision of matching the feed to the actual intake will significantly affect the repeatability of the analysis. Sampling of feces appeared to be less of a problem because of its higher AIA content (5.0% to 6.5% in feces), as noted by Van Keulen and Young (1977) and Sutton et al. (1977). However, small changes in acid insoluble ash concentration in the feces have a greater influence on the intake estimation calculation because the value is located in the denominator of the calculation equation  $(1 - AIA_{\text{diet}} / AIA_{\text{feces}}) \times 100$ . The AIA method has been performed in hay, concentrate, and mixed diets, in the absence of pasture. Because of the sensitivity of AIA estimation, grazing presents the opportunity for the voluntary ingestion of dirt or un-sampled herbage species that could lower the validity of the test.

### *1.7 e. Pasture Intake*

Pasture intake over a 24 hour period ranges from 1.5% to 3.1% BW in dry matter intake (DMI) or 15 to 32 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>, with the highest intakes seen in growing horses, lactating mares, and overconditioned ponies (NRC 2007). A summary of DMIs from mature, idle horses yielded a mean of 2.3% of BW from pasture (Dulphy et al. 1997, Fleurance et al. 2001, Chenost and Martin-Rosset 1985, Marlow et al. 1983, Chavez et al. 2014). Dugdale and colleagues (2010), over a 3 month introductory period, found that the intake rate of the overconditioned ponies was  $2.7 \pm 0.2\%$  of BW in DMI per day. Factors that influence DMI rate include herbage mass, physiological status of horse, forage quality, plant maturity, climate, plant species, digestibility, NSC content, crude protein content, and appetite (Dugdale et al. 2010, Glunk et al. 2012, Longland et al. 2006, Moffitt et al. 1987, Rogalski 1984). Collas and colleagues (2015) found that the DMI rate increased linearly (0.13 kg DM eaten/kg DW offered) with increasing herbage allowance.

Although the overconsumption of pasture should be closely monitored in obesity-prone horses, allowing horses access to pasture mimics normal foraging behavior, which reduces the likelihood of colic (Hudson et al. 2001) or gastric ulcers (Fleurance et al. 2001, Murray 1994). In addition, horses on pasture decrease the tendency to display stereotypical behaviors that are often seen in stalled horses (cribbing, weaving, and wind-sucking). Confined horses exhibit decreased bone development, as seen in growing colts. Stabled horses that suffer from chronic obstructive pulmonary disease may show remission of the disease when turned out on pasture (Derksen et al. 1985). Horses that have access to pasture have the necessary

means to exercise and play with pasture mates, boosting moral, physical condition, and overall well-being (Bott et al. 2013).

#### *1.7 f. Restricting Pasture in Obese Horses*

Restricting pasture intake by using a grazing muzzle or solitary confinement to a dry lot may be the best methods to control intake and prevent excess caloric intake in an overweight horse (Siciliano 2012). Restricting time at pasture as an attempt to decrease total pasture intake may be unsuccessful in some horses. Horses with restricted access to pasture have been known to increase DMI rate, which results in horses achieving 55% of maintenance DE requirement in 4 h of grazing (Dowler et al. 2012). This was similar to data collected by Ince and colleagues (2011), which concluded that horses can potentially eat up to 40% of their proposed DMI allotment (approximately 1.0% BW in DMI) within 3 h of grazing. Similarly, Longland and colleagues (2011) found that up to 1% of BW in dry matter can be ingested in ponies within 3 h. Glunk and colleagues (2013) found that restricting pasture access increased DMI rate during the first 4 hours of grazing by 67% of compared to the second 4 hour period. These findings are conclusive that horses and ponies tend to increase their intake rate when time at pasture is restricted. For horses grazing for less than 24 hours, Siciliano derived an equation that estimated DMI for horses with restricted access to pasture:  $y = 5.12x - 2.86$  ( $R^2 = 0.7$ ;  $p < 0.001$ ). According to this equation and previous study, horses with >12 h of pasture access may consume similar intakes to horses grazing for 24 h/d (Siciliano 2012).

Although grazing muzzles have shown success in restricting pasture intake, they present practical obstacles for horse owners, including opposition to use, trouble

maintaining proper fit, or trouble catching the horse to apply the grazing muzzle. In addition, their ability to efficiently restrict intake may depend on grass species and muzzle design (Glunk et al. 2013, 2014). Nevertheless, grazing muzzles provide the opportunity for equines to engage in activity at pasture while intake rate is restricted. The amount of pasture restriction that grazing muzzles provide has been investigated with mixed results. Longland and coworkers (2011) found that reduction in DMI for spring, summer, and autumn, was 77%, 78%, and 83% of un-muzzled intake rate over 3 h. However, Van Weyeneberg et al. (2008) found that grazing muzzles reduced the amount of grass ingested by 25 to 33% (Van Weyenberg et al. 2008), which was similar to results by Glunk and colleagues (2014) (40%).

#### *1.7 g. Pasture and Insulin Resistance*

Grazing horses may develop insulin resistance as a chronic adaptation to high simple sugar, fructan, and starch content (Harris et al. 2011, Johnson et al. 2010, Longland et al. 2006, Treiber et al. 2006). Spring pasture non-structural carbohydrate (NSC) concentrations can constitute 25% to 30% of dry matter content (Bailey et al. 2013, Longland et al. 2006). Furthermore, peaks in insulin concentration can occur following a sudden increase in pasture availability and intake. Horses prefer sweet, high NSC forage, and an increase in NSC rich grass is oftentimes seen during the spring (May and June). McIntosh and colleagues (2007) found that mean plasma insulin concentrations in horses with 24-h access to pasture was highest ( $54.6 \pm 9.9 \mu\text{U/mL}$  in vs. control  $11.7 \pm 3.4 \mu\text{U/mL}$  ( $P < 0.001$ ) in April (McIntosh et al. 2007). Pastures undergo daily and seasonal fluctuations in WSC content (Undersander 2013). NSC and fructan content tends to be lower in warm season grass varieties than cool season

grasses, confirmed by observations of 129 g and 209 g NSC/kg DM, respectively, by Chaterton and coworkers (1989). The NSC concentration of cool season pasture grasses rises during daylight hours and fall overnight (Kagan et al. 2011, Wyckoff et al. 2013). Overall, NSC level is highly influenced the growing conditions (i.e., fertilization, defoliation, and irrigation), pasture height (Siciliano and Gill 2016), and climate-related factors pertaining to location, all of which affect insulin concentration in grazing horses (Treiber et al. 2006). High levels of NSC may contribute to a higher amount of digestible energy in the forage, which should be avoided in idle, overweight horse. Additionally, forages containing high NSC content may support the development of IR and metabolic deficiencies in susceptible horses (Hoffman et al. 2003, NRC 2007).

#### *1.7 h. Activity at Pasture*

The vast majority of activity at pasture is grazing and resting. It has been observed that grazing behavior varies depending upon a number of factors (i.e., hours of daylight, season of the year, temperature, climate, *etc.*). Horses maintained at pasture have been observed to graze 75% of the day and as much as 50% of the evening hours (Fleurance et al. 2001, Frape 2004, Lewis 1995). Horses increase their frequency of grazing in the early morning, late afternoon and evening, and the middle of the night (Lewis 1995). High temperatures and heavy rainfall decrease time spent grazing (Crowell-Davis et al. 1985, Houpt 1990), whereas cold conditions or high relative humidity increase grazing time (Rogalski 1974). In addition, horses decrease grazing in hot weather by approximately 15% to 20% (Booth 1998, Cymbaluk and Christison 1990). On average, domesticated horses travel 2.6 to 5.0 km/d (Booth 1998, Shingu et al. 2000, NRC 2007), with an estimated 50% to 60% of activity occurring while grazing.

Pasture availability is positively associated with time spent grazing (Arnold 1984, NRC 2007). With decreasing pasture supply, horses decreased both pasture intake (1.8 to 2.0 kg DM·kg BW<sup>-1</sup>·d<sup>-1</sup>) and time spent grazing (52 to 58%; Shingu et al. 2000).

Confinement in horses and abnormal feeding patterns may facilitate obesity in horses (Rivera et al. 2002). Thus, grazing on ample pasture may mimic feral horse conditions, instituting a healthier, more active lifestyle. Pasture access has been shown to increase the ability of a horse to maintain muscle, bone mineral content, and exercise fitness ability (Bell et al. 2001, Graham-Thiers and Bowen 2013). Both the size and shape of the pasture area can affect the behavior of horses at pasture. Hampson and colleagues (2010) found a logarithmic increase in mean daily distance traveled with increasing pasture size. In addition they found that feral horses travel substantially farther distances (17.9 km/d) than domesticated mares (7.2 km/d in a 16-ha pasture) (Hampson et al. 2010).

### *1.8 Energy Expenditure*

The amount of energy expenditure must be known to estimate the DE expended during exercise. The amount of energy utilized by exercise is a function of the duration and intensity. Oxygen consumption during exercise has been used as a proxy for estimating energy expenditure in horses. Several studies have found that there is a linear relationship between oxygen utilization and speed (Eaton et al. 1995, Hiraga et al. 1995). Factors influencing oxygen utilization from speed include the level of warm-up (McCutcheon et al. 1999, Tyler et al. 1996), conditioning (Eaton et al. 1999), and body composition (Kearns et al. 2002). Thus, the precision of estimating energy expenditure from speed is subject to considerable within-horse variation. In some disciplines (i.e.,

cutting, gaited events), the relationship between oxygen utilization and speed may be irrelevant, and therefore may not accurately predict energy costs. Because there is a strong relationship between heart rate during exercise and oxygen utilization, it is possible to estimate energy expenditure from HR (Coenen 2005, Eaton et al. 1995). Oxygen utilization in horses is more closely related to maximal HR than actual HR because of differences in fitness levels between horses, but it is hard to elicit these responses in most exercising horse. Nevertheless, average heart rate reflects the relative intensity of overall workload. The availability of heart rate monitoring devices has made it possible to easily measure energy expenditure in horses during field training, without the use of a treadmill. In this manner, the energy cost of an actual exercise program can be assessed. Kingston and colleagues (2006) found that a Garmin GPS/HR monitoring system provided a reliable measure of training intensity of exercise during race training. Coenen (2005) summarized oxygen consumption data from 87 studies and developed the following equation to estimate energy expenditure from HRs between 60 to 180 bpm:

$$\text{Oxygen Utilization (mL O}_2\text{/kg BW/min)} = 0.0019 \times (\text{HR})^{2.0653} \text{ (R}^2 = .90\text{)}$$

In conjunction with the energy equivalent of 1 L of O<sub>2</sub> (4.86 kcal), it is possible to determine the energy utilization of exercise in kcal/min. The cost of exercise is then added to the maintenance energy requirement to determine the DE requirement for working horses. The NRC lists the four categories of working horses as: light, moderate, heavy, and very heavy. The percentages of DE above maintenance in ascending order are 20%, 40%, 60% and 90%, respectively.

Most research has measured energy expenditure as it pertaining to work, *i.e.*, energy in excess of maintenance requirements. However, there is a deficit of knowledge pertaining to the voluntary energy expenditure value associated with pasture access. Estimating energy expenditure in grazing animals is important for management purposes. Heat production (HP) is related to energy expenditure and is affected by various environmental, digestive, and activity-related factors. In previous research, relating to the development and evaluation of metabolic energy expenditure in horses, HP has been essential in estimating energy costs. Yamamoto (1989) evaluated the relationship between HP and HR in free living farm animals. The author used the proxy, relative heart rate (RHR), the ratio of actual HR to basal HR, to eliminate individual and day-day variation for a group of animal species. The author cautioned that it is not possible to estimate the energy efficiency of production in grazing animals unless the daily dry matter intake is known.

Horses that are idle or used for leisure activities have different nutritional requirements than horses used for performance or production. The management of horses with lower energy requirements involves careful monitoring of digestible energy intake and modification of husbandry practices, especially during the spring and summer when the pasture supply and DE content are the greatest to prevent obesity. The combination of over-nutrition and inadequate physical activity has increased the prevalence of obesity and related metabolic disorders in horses. Dietary management in obese horses requires the combination of decreased calorie intake and increased energy expenditure, and required strict control on dietary intake and the successful

implementation of a feeding regimen. Pasture presents a dietary commodity where voluntary intake cannot be easily measured or controlled. Common methods of restricting pasture intake include time-restricted grazing or the use of grazing muzzles, which have been shown to moderately decrease forage consumption but also result in the underutilization of forage. Managing horses at pasture increases energy expenditure compared to stall confinement, which maintains fitness, elevates mood, and decreases stereotypical behaviors. Because a majority of horses are managed solely on pasture, methods for achieving an optimal body condition and sound metabolic profile in grazing horses is warranted.

### *Objectives*

Obesity in horses is a serious condition with risks that include insulin resistance, equine metabolic syndrome, and laminitis. It is recognized that the reversal of obesity can be carried out in an experimental setting, resulting in reduced adiposity and insulin concentration. However, individual variation in weight loss responsiveness has been noted, classifying some horses and ponies as 'diet resistant'. It has been recognized that horses differ in energy requirement for maintenance according to turnout (voluntary activity) and metabolism, both of which require further quantification. The underlying cause of resistance to weight loss may be attributed to lower maintenance requirements because of reduced voluntary activity and/or variability in metabolism (as seen in humans). In addition, no research has been done to the author's knowledge to quantify the difference (if any) in voluntary pasture intake between obese and moderate conditioned horses. It is possible that the combination of overeating,

inactivity, and a low metabolism sets the prime scenario for obesity and disease prevalence.

An objective of this research was to assess the effectiveness of weight loss protocols carried out by horse owners and caretakers and evaluate resting insulin concentration upon weight reduction. In addition, individual variability among horses, with respect to calorie intake, change in BW/BCS, and activity level, were evaluated to identify horses with increased risk for obesity. Because pasture is a contributing factor for obesity and disease incidence in horses, an objective was to limit pasture intake by means of spatial and time restriction for the reduction of BW, BCS, and INS concentration. EXPT 1 was conducted to determine if weight loss in overweight horses can be achieved by their owners when feed is restricted to 1.25% to 1.50% of BW/d and 80% to 90% of maintenance DE requirement. The objective of the EXPT 2 was to perform a retrospective analysis of the relationships among DE intake, BW, and BCS in lean/moderate condition horses fed above maintenance DE requirements. The objective of EXPT 3 was to quantify individual variation in DMI and activity between horses at pasture. The objective of EXPT 4 was to determine if horses would decrease in BW, BCS, and basal INS if the pasture herbage allowance was restricted to 80% of the maintenance DE requirements. Additionally, differences in activity between 24-h and 8-h of grazing were accessed.

Table 1: Henneke Body Condition Scoring (BCS) System for Horses

| Body Condition Score | Name            | Description  |
|----------------------|-----------------|--|
| 1                    | Poor/emaciated  | Body structures of neck, shoulders and withers easily noticeable. Spinous process along the ribs, topline, point of hip and point of buttocks prominent. Significant space between the inner buttocks. No fatty tissue can be felt.  |
| 2                    | Very thin       | Bony structures of neck, shoulders, and withers are faintly discernible. Spinous processes, ribs, topline, point of hip and point of buttock are prominent. Noticeable space between inner buttocks.   |
| 3                    | Thin            | Neck, withers, and shoulder are accentuated, but not obviously thin. Tailhead is prominent. Slight fat covering over ribs, but easily discernible. Spinous process, point of hip, and point of buttock are rounded, but easily discernible.  |
| 4                    | Moderately-thin | Neck, withers and shoulders are not obviously thin. Ribs are faintly discernible. Point of hip and point of buttock are not visually discernible. Fat can be felt around the tailhead. Slight negative crease along the topline, especially over the loins and hindquarters.                       |
| 5                    | Moderate        | Neck withers and shoulder appear rounded and blend smoothly into the body. Ribs cannot be seen, but are easily felt. Back is level with neither a ridge nor a positive crease along the topline. Fat around tailhead is beginning to feel spongy. Slight amount of fat deposited between buttocks. |
| 6                    | Moderate-fleshy | Fat beginning to be deposited along the neck, withers, and shoulders. Fat over the ribs is beginning to feel spongy. Ribs cannot be easily felt. Fat around tailhead feels soft. May be a slight positive crease along the topline. Noticeable fat deposition between buttocks.                    |
| 7                    | Fleshy          | Fat deposition along neck and withers and behind shoulder. Individual ribs can be felt, but with noticeable filling between the ribs. Slight positive crease down the back. Fat around the tailhead feels soft.  |
| 8                    | Fat             | Noticeable thickening of neck. Area along withers is filled with fat. Area behind shoulder is filled in flush with body. Ribs cannot be felt. Noticeable positive crease down back. Fat around tailhead is very soft. Significant fat deposited along inner buttocks.                              |
| 9                    | Extremely fat   | Bulging fat along neck, shoulders, and withers. Flanks are filled in and flush with body. Patchy fat appearing over ribs. Obvious positive crease down back. Obvious fat deposited along inner buttocks.   |

Table 2: Overweight Body Condition Score Statistics from the US and UK

| Study                        | Location           | Sample Size | Body Condition Scoring Method | Observer       | Definition of Obese | Overweight (%) | Obese (%) |
|------------------------------|--------------------|-------------|-------------------------------|----------------|---------------------|----------------|-----------|
| Thatcher et al. (2008)       | Virginia           | 300         | Henneke et al. (1983)         | Professionals  | BCS $\geq$ 7.5      | 32             | 19        |
| Wyse et al. (2008)           | Scotland (UK)      | 319         | Webb and Weaver (1979)        | Owner-reported | BCS $>$ 3/5         | 20.6           | -         |
| Pratt-Phillips et al. (2010) | North Carolina     | 366         | Henneke et al. (1983)         | Professionals  | BCS $\geq$ 7/9      | 48             | 20        |
| Harker et al. (2011)         | England (UK)       | 331         | Henneke et al. (1983)         | Professionals  | BCS $\geq$ 7/9      | 41             | 21        |
| Robin et al. (2015)          | Great Britain (UK) | 792         | Carroll and Huntington (1988) | Owner-reported | BCS $\geq$ 5/6      | -              | 31        |
| Martin-Gomez et al. (2016)   | South-East Spain   | 127         | Henneke et al. (1983)         | Professionals  | BCS $\geq$ 7/9      | 82             | 26        |

Table 3. Weight Loss Studies in Horses: Change in Body Weight ( $\Delta$ BW), Body Condition Score ( $\Delta$ BCS), and Insulin Concentration ( $\Delta$ INS)

| Authors                     | Animals              | Initial BCS      | Duration                       | Restriction  | $\Delta$ BW (%)                        | $\Delta$ BCS   | $\Delta$ INS                   |
|-----------------------------|----------------------|------------------|--------------------------------|--|--|----------------|--------------------------------|
| McGowan et al. (2013)       | 12 horses            | 7.6 $\pm$ 0.7/9  | 6 weeks                        | 1.25% BW/day DM, 65% DE Req.                         | 6.8%                                   | 0.4 $\pm$ 0.75 | 4.8 mIU/L                      |
| Argo et al. (2012)          | 12 horses and ponies | 7.8 $\pm$ 0.18/9 | 16 weeks, extended to 20 weeks | 1.25% BW/day in DM, reduced to 1% for 4 wks (GE 50%) | 6.88 $\pm$ 1.75% and 7.39% $\pm$ 1.10% | 1.5 $\pm$ 0.3  | 75% of animals saw improvement |
| Dugdale et al. (2010)       | 5 ponies             | 6.8 $\pm$ 0.5/9  | 12 weeks                       | 1% of BW/day, 67% DE Req.                            | 11.4 $\pm$ 1.9%                        | 0.3 $\pm$ 0.14 | 37.89 mU/L                     |
| Van Weyenberg et al. (2007) | 9 ponies             | $>$ 8/9          | 18 weeks                       | 70% DE Req. (wks 1-5), 50% (6-13), 35% (14-18)       | 18.2 $\pm$ 1.8% BM                     | 4.0 $\pm$ 1.0  | 9.6 mU/L (50% reduction)       |



| Score | Description   |
|-------|---|
| 0     | No palpable crest.  |
| 1     | No visual appearance of a crest, but slight filling felt with palpation.  |
| 2     | Noticeable appearance of a crest, but fat deposited fairly evenly from poll to withers. Crest easily cupped in one hand and bent from side to side.   |
| 3     | Crest enlarged and thickened, so fat is deposited more heavily in middle of the neck than toward poll and withers, giving a mounded appearance. Crest fills cupped hand and begins losing side to side flexibility. |
| 4     | Crest grossly enlarged and thickened, and can no longer be cupped in one hand or easily bent from side to side. Crest may have wrinkles or creases perpendicular to the topline.                                    |
| 5     | Crest is so large it permanently droops to one side.  |

**Figure 1.** Cresty neck scoring in horses (Carter et al. 2008)

## Literature Cited

- Becvarova I., and R. S. Pleasant. 2012. Managing obesity in pasture-based horses. Vetlearn - Continuing Education for Veterinarians, e1-4.
- Carroll, C. L., and P. J. Huntington. 1988. Body condition scoring and weight estimation in horses. *Equine Vet. J.* 20(1): 41-45.
- Carter, R. A., and A. H. Dugdale. 2012. Assessment of body condition and bodyweight. *Appl. Nutr.* Chapter 22.
- Dugdale, A. H., C. Curtis, P. Cripps, P. A. Harris, C. and McG. Argo. 2011a. Effect of season and body condition on appetite, body mass and body composition in ad libitum fed pony mares. *Vet. J.* 190(3): 329-337.
- Dugdale, A. H., G. C. Curtis, P. Cripps, P. A. Harris, and C. McG. Argo. 2010. Effects of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Vet. J.* 42(7): 600-610.
- Dugdale, A. H., G. C. Curtis, P. A. Harris, and C. McG. Argo. 2011b. Assessment of body fat in the pony: I. Relationships between the anatomical distribution of adipose tissue, body composition and body condition. *Equine Vet. J.* 43(5): 552-562.
- Dugdale, A. H., G. C. Curtis, P. J. Cripps, P. A. Harris, C. McG. Argo. 2011c. Assessment of body fat in the pony: II. Validation of the deuterium oxide dilution technique for the measurement of body fat. *Equine Vet. J.* 43(5): 562-570.
- Frank, N., R. J. Geor, S. R. Bailey, A. E. Durham, and P. J. Johnson. 2010. Equine Metabolic Syndrome. *J. Vet. Inter. Med.* 24(3): 467-475.
- Gentry, L. R., D. L. Thompson, G. T. Gentry Jr, K. A. Davis, R. A. Godke, J. A. Cartmill. 2002. The relationship between body condition, leptin, ad reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J. Anim. Sci.* 80(10): 2695-2703.
- Henneke, D. R., G. D. Potter, J. L. Kreider, B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15(4): 371-372.
- Kohnke, J. 1992. *Feeding and nutrition: The making of a champion*. Birubi Pacific, Australia, 163-166.
- Laflamme, D. 1997. Development and validation of a body condition scoring system for dogs. *Canine Prac.* 22: 10-15.

- Suagee, J. K., A. O. Burk, R. W. Quinn, E. D. Petersen, T. G. Hartsock, L. W. Douglass. 2008. Effects of diet and weight gain on body condition scoring in Thoroughbred geldings. *J. Equine Vet. Sci.* 28(3): 156-166.
- Van der Aa Kuhle, K., A. J. Cawdell-Smith, L. C. Ward and W. L. Bryden. 2008. Prediction of equine body composition with bioelectrical impedance spectroscopy. *Proc. Nutr. Soc. Austr.* 17(3): S43.
- Vick, M. M., D. R. Sessions, B. A. Murphy, E. L. Kennedy, S. E. Reedy, and B. P. Fitzgerald. 2006. Obesity associated with altered metabolic and reproductive activity in the mare: effects of Metformin on insulin sensitivity and reproductive cyclicity. *Equine Vet. J.* 18(6): 609-617.
- Mawby, D. I., J. W. Bartges, A. d'Avignon, D. P. Laflamme, T. D. Moyers, T. Cottrell. 2004. Comparison of various methods for estimating body fat in dogs. *J. Amer. Anim. Hosp. Assoc.* 40(2): 109-114.
- Martinson, K. L., R. C. Coleman, A. K. Rendahl, Z. Fang, M. E. McCue. 2014. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. *J. Anim. Sci.* 92(5): 2230-2238.
- Martin-Gimenez, T., C. N. Aguirre-Pascasio, I. de Blas. 2016. Ultrasonographic assessment of regional fat distribution and its relationship with body Condition in an easy keeper horse breed. *J. Equine Vet. Sci.* 39: 69-75.
- Garlinghouse, S. E., and M. J. Burrill. 1999. Relationship of body condition score to completion rate during 160 km endurance races. *Equine Vet. J.* 31(S30): 591-595.
- Cavinder, C. A., M. M. Vogelsang, D. W. Forrest, P. G. Gibbs, and T. L. Blanchard. 2005. Reproductive parameters of fat vs moderately conditioned mares following parturition. In *Proc. 19th Equine Sci. Soc. Symp.* 65-70.
- Catalano, D. N., R. J. Coleman, M. R. Hathaway, M. E. McCue, A. K. Rendahl, and K. L. Martinson. 2015. Estimation of actual and ideal bodyweight using morphometric measurements and owner guessed bodyweight of adult draft and warmblood horses. *J. Equine Vet. Sci.* (2015).
- Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *J. Anim. Sci.* 81(9): 2333-2342.
- Carter, R. A., L. J. McCutcheon, L. A. George, T. L. Smith, N. Frank, and R. J. Geor. 2009. Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses. *Amer. J. Vet. Res.* 70(10): 1250-1258.

- Treiber, K. H., D. S. Kronfeld, T. M. Hess, B. M. Byrd, R. K. Splan, and W. B. Stanier. 2006. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *J. Amer. Vet. Med. Assoc.* 228(10): 1538-1545.
- Geor, R. J. 2008. Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *J. Equine Vet. Sci.* 28(12): 753-759.
- Vick, M. M., A. A. Adams, B. A. Murphy, D. R. Sessions, D. W. Horohov, R. F. Cook, B. J. Shelton, and B. P. Fitzgerald. 2007. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. *J. Anim. Sci.* 85(5): 1144-1155.
- Carter, R. A. 2008. Equine obesity and its role in insulin resistance, inflammation and risk for laminitis. Doctoral dissertation, Virginia Polytechnic Institute and State University.
- Buff, P. R., A. C. Dodds, C. D. Morrison, N. C. Whitley, E. L. McFadin, J. A. Daniel, and D. H. Keisler. 2002. Leptin in horses: Tissue localization and relationship between peripheral concentrations of leptin and body condition. *J. Anim. Sci.* 80(11): 2942-2948.
- Freesone, J. F., K. Shoemaker, R. Bessin, and K. J. Wolfsheimer. 1992. Insulin and glucose response following oral glucose administration in well-conditioned ponies. *Equine Vet. J.* 24 (Suppl. 11):13-17.
- Kane, R. A., M. Fisher, D. Parrett, and L. M. Lawrence. 1987. Estimating fatness in horses. 127-131. *Proc. 10<sup>th</sup> Equine Nutr. Physiol. Symp.*, Ft. Collins, CO.
- Bianchi, M. 1989. Fat cell size in various body regions. A statistical analysis in *Equus caballus*. *Anat. Anz. Jena.* 169(5): 351-366.
- Fitzgerald, B. P., S. E. Reedy, D. R. Sessions, M. M. Vick, and B. A. Murphy. 2003. Obesity disrupts the duration of the estrous cycle in the mare. *J. Anim. Sci.* 81(Suppl. 1): 102.
- Kearns, C. F., K. H. McKeever, and T. Abe. 2002. Overview of horse body composition and muscle architecture: Implications for performance. *Vet. J.* 164(3): 224-234.
- Potter, D. M., J. W. Evans, G. W. Webb, and S. P. Webb. 1987. Digestible energy requirements of Belgian and Percheron horses. *Proc. 11<sup>th</sup> Equine Nutr. Physiol. Symp.* Fort Collins, CO. 133-138.
- Webb, A. I., and B. M. Q. Weaver. 1979. Body composition of the horse. *Equine Vet. J.* 11(1): 39-47.
- Westervelt, R. G., J. R. Stouffer, H. F. Hintz, and H. F. Schryver. 1976. Estimating fatness in horses and ponies. *J. Anim. Sci.* 43(4):781-785.

- Quinn, R. W. 2007. Effect of weight gain, diet and exercise on insulin sensitivity in Thoroughbred geldings. *ProQuest*.
- Kubiak, J. R., B. H. Crawford, E. L. Squires, R. H. Wrigley, and G. M. Ward. 1987. The influence of energy intake and percentage of body fat on the reproductive performance of nonpregnant mares. *Theriogenology* 28(5): 587-598.
- Owen, G. S., E. L. Wagner, and W. S. Eller. 2008. Estimation of body weight in ponies. *J. Anim. Sci.* 86: 431.
- Kienzle, E., and S. C. Schramme. 2004. Body condition scoring and prediction of body weight in adult warm blooded horses. *Pferdeheilkunde* 20(6): 517-524.
- Lawrence, L. M., N. F. Cymbaluk, D. W. Freeman, R. J. Geor, P. M. Graham-Thiers, A. C. Longland, et al. 2007. Nutrient requirements of horses. 6th ed. The National Academies Press, Washington DC.
- Johnson, P. J., S. H. Slight, V. K. Ganjam, and J. M. Kreeger. 2002. Glucocorticoids and laminitis in the horse. *Vet. Clinics N. Amer.: Equine Pract.* 18(2): 219-236.
- King, C., and R. A. Mansmann. 2004. Preventing laminitis in horses: dietary strategies for horse owners. *Clin. Techn. Equine Pract.* 3(1): 96-102.
- Scheibe, K. M., and W. J. Streich. 2003. Annual rhythm of body weight in Przewalski horses (*Equus ferus przewalskii*). *Bio. Rhythm Res.* 34(4): 383-395.
- de Laat, M. A., C. M. McGowan, M. N. Sillence, and C. C. Pollitt. 2010. Equine laminitis: Induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine Vet. J.* 42(2): 129-135.
- Johnson, P. J., C. E. Wiedmeyer, A. LaCarrubba, V. S. Ganjam, N. T. Messer. 2010. Laminitis and the equine metabolic syndrome. *Vet. Clin. N. Amer.: Equine Pract.* 26(2): 239-255.
- American Horse Council. 2005. Deloitte Consulting LLP. National Economic Impact on the US Horse Industry. Washington.
- Giles, S. L., S. A. Rands, C. J. Nicol, P. A. Harris. 2014. Obesity prevalence and associated risk factors in outdoor living domestic horses and ponies. *PeerJ* 2, e299.
- Robin, C. A., J. L. Ireland, C. E. Wylie, S. N. Collins, K. L. P. Verheyen, and J. R. Newton. 2015. Prevalence of and risk factors for equine obesity in Great Britain based on owner-reported body condition scores. *Equine Vet. J.* 47(2): 196-201.

- Thatcher, C. D., R. S. Pleasant, R. J. Geor, F. Elvinger. 2012. Prevalence of overconditioning in mature horses in Southwest Virginia during the summer. *J. Vet. Intern. Med.* 26(6): 1413-1418.
- Pratt-Phillips, S. E., K. M. Owens, L. E. Dowler, M. T. Cloninger. 2010. Assessment of resting insulin and leptin concentrations and their association with managerial and innate factors in horses. *J. Equine Vet. Sci.* 30(3): 127-133.
- Wyse, C. A., K. A. McNie, V. J. Tannahil, J. K. Murray, and S. Love. 2008. Prevalence of obesity in riding horses in Scotland. *Vet. Record* 162(18): 590.
- Harker, I. J., P. A. Harris, and C. F Barfoot. 2011. The body condition score of leisure horses competing at an unaffiliated championship in the UK. *J. Equine Vet. Sci.* 31(5): 253-254.
- Rohlf, V. I., S. Toukhsati, G. J. Coleman, and P. C. Bennett. 2010. Dog obesity: Can dog caregivers (owners) feeding and exercise intentions and behaviors be predicted from attitudes? *J. Appl. Anim. Welfare Sci.* 13(3): 213-236.
- Geor, R. J. 2010. Current concepts on the pathophysiology of pasture-associated laminitis. *Vet. Clinics N. Amer. Equine Pract.* 26(2): 265-276.
- Cymbaluk, N. F., and G. I. Christison. 1990. Environmental effects on thermoregulation and nutrition of horses. *Vet. Clin. North Am. Equine Pract.* 6(2): 355-372.
- Johnson, P. J., C. E. Wiedmeyer, A. LaCarrubba, V. S. Ganjam, and N. T. Messer. 2012. Diabetes, insulin resistance, and metabolic syndrome in horses. *J. Diabetes Sci. Technol.* 6(3): 534-540.
- Webb, S. P., G. D. Potter, J. W. Evans, and G. W. Webb. 1989. Influence of body fat content on digestible energy requirement of exercising horses in temperate and hot environments. 279-284. *Proc. 11<sup>th</sup> Equine Nutr. Physiol. Symp., Stillwater, OK.*
- Geor, R. J. 2009. Pasture-associated laminitis. *Vet. Clinics N. Amer. Equine Pract.* 25(1): 39-50.
- Vick, M. M., B. A. Murphy, D. R. Sessions, S. E. Reedy, E. L. Kennedy, D. W. Horohov, and B. P. Fitzgerald. 2008. Effects of systemic inflammation on insulin sensitivity in horses and inflammatory cytokine expression in adipose tissue. *Amer. J. Vet. Res.* 69(1): 130-139.
- Carter, R. A., R. J. Geor, W. B. Staniar, T. A. Cubitt, and P. A. Harris. 2009. Apparent adiposity assessed by standardized scoring systems and morphometric measurements in horses and ponies. *Vet. J.* 179(2): 204-210.

- Harris, P., S. R. Bailey, J. Elliott, and A. Longland. 2006. Countermeasures for pasture-associated laminitis in ponies and horses. *J. Nutr.* 136(7): 2114S-2121S.
- Suagee, J. K., A. O. Burk, R. W. Quinn, T. G. Hartsock, and L. W. Douglass. 2011. Effects of diet and weight gain on circulating tumor necrosis factor- $\alpha$  concentrations in Thoroughbred geldings. *J. Anim. Physiol. Anim. Nutr.* 95(2): 161-170.
- Asplin, K. E., M. N. Sillence, C. C. Pollitt, and C. M. McGowan. 2007. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *Vet. J.* 174(3): 530-535.
- McGowan, C. M., A. H. Dugdale, G. L. Pinchbeck, and C. M. Argo. 2013. Dietary restriction in combination with a nutraceutical supplement for the management of equine metabolic syndrome in horses. *Vet. J.* 196(2): 153-159.
- Bailey, S. R., C. M. Marr, and J. Elliott. 2004. Current research and theories on the pathogenesis of acute laminitis in the horse. *Vet. J.* 167(2): 129-142.
- Bailey, S. R., J. L. Habershon-Butcher, K. J. Ransom, J. Elliott, and N. J. Menzies-Gow. 2008. Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis. *Amer. J. Vet. Res.* 69(1): 122-129.
- Borer, K. E., S. R. Bailey, N. J. Menzies-Gow, P. A. Harris, and J. Elliott. 2003. Effect of feeding glucose, fructose, and inulin on blood glucose and insulin concentrations in normal ponies and those predisposed to laminitis." *J. Anim. Sci.* 90(9): 3003.
- Undersander, D. 2013. Grass varieties for horses. *J. Equine Vet. Sci.* 33(5), 315-320.
- Daly, K., C. J. Proudman, S. H. Duncan, H. J. Flint, J. Dyer, and S. P. Shirazi-Beechey. 2012. Alterations in microbiota and fermentation products in equine large intestine in response to dietary variation and intestinal disease. *Br. J. Nutr.* 107(7): 989-995.
- Julliand, V., A. De Fombelle, and M. Varloud. 2006. Starch digestion in horses: The impact of feed processing. *Livest. Sci.* 100(1): 44-52.
- Al Jassim, R. A., and F. M. Andrews. 2009. The bacterial community of the horse gastrointestinal tract and its relation to fermentative acidosis, laminitis, colic, and stomach ulcers. *Vet. Clinics N. Amer. Equine Pract.* 25(2): 199-215.
- Milnovich, G. J., D. J. Trott, P. C. Burrell, A. W. Van Eps, M. B. Thoenes, L. L. Blackall, and C. C. Pollitt. 2006. Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. *Environ. Microbiol.* 8(5): 885-898.
- McGowan, C. M., R. Geor, and T. W. McGowan. 2008. Prevalence and risk factors for hyperinsulinemia in ponies. *J. Vet. Intern. Med.* 22(3): 734-734.

Burns, T. A., M. R. Watts, P. S. Weber, L. J. McCutcheon, R. J. Geor, and J. K. Belknap. 2015. Laminar inflammatory events in lean and obese ponies subjected to high carbohydrate feeding: Implications for pasture-associated laminitis. *Equine Vet. J.* 47(4): 489-493.

Bailey, S. R., and N. J. Bamford. 2013. Metabolic responses of horses and ponies to high and low glycaemic feeds: implications for laminitis. *Animal Prod. Sci.* 53(11): 1182-1187.

McCue, M. E., R. J. Geor, and N. Schultz. 2015. Equine metabolic syndrome: A complex disease influenced by genetics and the environment. *J. Equine Vet. Sci.* 35(5): 367-375.

Frank, N. 2006. Insulin resistance in horses. *AAEP Proc.* 52: 51-54.

Cluny, N. L., R. A. Reimer, and K. A. Sharkey. 2012. Cannabinoid signaling regulates inflammation and energy balance: the importance of the brain-gut axis. *Brain Behav. Immun.* 26(5): 691-698.

Treiber, K., R. Carter, L. Gay, C. Williams, and R. Geor. 2009. Inflammatory and redox status of ponies with a history of pasture-associated laminitis. *Vet. Immunol. Immunopathol.* 129(3): 216-220.

Bastard, J-P., M. Mustapha, J. Tran van Nhieu, C. Jardel, E. Bruckert, A. Grimaldi, J-J. Robert, J. Capeau, and B. Hainque. 2002. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *J. Clinical Endocrinol. Metabol.* 87(5): 2084-2089.

Adams, A. A., M. P. Katepalli, K. Kohler, S. E. Reedy, J. P. Stilz, M. M. Vick, B. P. Fitzgerald, L. M. Lawrence, and D. W. Horohov. Effect of body condition, body weight and adiposity on inflammatory cytokine responses in old horses. *Vet. Immunol. Immunopathol.* 127(3): 286-294.

Burns, T. A., Geor, R. J., Mudge, M. C., McCutcheon, L. J., Hinchcliff, K. W., and J. K. Belknap. 2010. Proinflammatory cytokine and chemokine gene expression profiles in subcutaneous and visceral adipose tissue depots of insulin-resistant and insulin-sensitive light breed horses. *J. Vet. Intern. Med.* 24(4): 932-939.

Frank, N., S. B. Elliott, L. E. Brandt, and D. H. Keisler. 2006. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J. Amer. Vet. Med. Assoc.* 228(9): 1383-1390.

Pratt-Phillips, S. E., K. M. Owens, L. E. Dowler, and M. T. Cloninger. 2010. Assessment of resting insulin and leptin concentrations and their association with managerial and innate factors in horses. *J. Equine Vet. Sci.* 30(3): 127-133.

Stewart-Hunt, L., S. Pratt-Phillips, L. J. McCutcheon, and R. J. Geor. 2010. Dietary energy source and physical conditioning affect insulin sensitivity and skeletal muscle glucose metabolism in horses. *Equine Vet. J.* 42(s38): 355-360.

Bamford, N. J., S. J. Potter, P. A. Harris, and S. R. Bailey. 2014. Breed differences in insulin sensitivity and insulinemic responses to oral glucose in horses and ponies of moderate body condition score. *Domest. Anim. Endocrinol.* 47: 101-107.

Vermorel, M., W. Martin-Rosset, and J. Vernet. 1997. Energy utilization of twelve forages or mixed diets for maintenance by sport horses. *Livest. Prod. Sci.* 47(2): 157-167.

Pagan, J. D., and H. F. Hintz. 1986. Equine energetics. I. Relationship between body weight and energy requirements in horses. *J. Anim. Sci.* 63(3): 815-821.

Kubiak, J. R., J. W. Evans, G. D. Potter, P. G. Harms, and W. L. Jenkins. 1988. Parturition in the multiparous mare fed to obesity. *J. Equine Vet. Sci.* 8(2): 135-140.

Frape, D. 2008. *Equine nutrition and feeding.* John Wiley & Sons.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *J. Endocrinol.* 165(2): 519-526.

Marsh, D. J., G. Hollopeter, K. E. Kafer, and R. D. Palmiter. 1998. Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nature Med.* 4(6): 718-721.

Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, and S. Matsukura. 2001. A role for ghrelin in the central regulation of feeding. *Nature* 409(6817): 194-198.

Wren, A. M., L. J. Seal, M. A. Cohen, A. E. Brynes, G. S. Frost, K. G. Murphy, W. S. Dhillo, M. A. Ghatei, and S. R. Bloom. 2001. Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* 86(12): 5992.

Shiia, T., M. Nakazato, M. Mizuta, Y. Date, M. S. Mondal, M. Tanaka, S-I. Nozoe, H. Hosoda, K. Kangawa, and S. Matsukura. 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J. Clin. Endocrinol. Metab.* 87(1): 240-244.

Bailey, S. R., and N. J. Bamford. 2013. Metabolic responses of horses and ponies to high and low glycaemic feeds: Implications for laminitis. *Anim. Prod. Sci.* 53(11): 1182-1187.

Kronfeld, D. S. 1993. Starvation and malnutrition of horses: Recognition and treatment. *J. Equine Vet. Sci.* 13(5): 298-304.

Pratt, S. E., R. J. Geor, and L. J. McCutcheon. 2006. Effects of dietary energy source and physical conditioning on insulin sensitivity and glucose tolerance in Standardbred horses. *Equine Vet. J.* 38(S36): 579-584.

Pratt-Phillips, S., J. Kutzner-Mulligan, R. Marvin, H. Brown, C. Sykes, and J. Federico. 2014. The effect of feeding two or three meals per day of either low or high nonstructural carbohydrate concentrates on postprandial glucose and insulin concentrations in horses. *J. Equine Vet. Sci.* 34(11): 1251-1256.

Quinn, R. W., A. O. Burk, T. G. Hartsock, E. D. Petersen, N. C. Whitley, K. Treiber, and R. C. Boston. 2008. Insulin sensitivity in Thoroughbred geldings: Effect of weight gain, diet, and exercise on insulin sensitivity in Thoroughbred geldings. *J. Equine Vet. Sci.* 28(12): 728-738.

Kronfeld, D. S., K. H. Treiber, T. M. Hess, and R. C. Boston. 2005. Insulin resistance in the horse: Definition, detection, and dietetics. *J. Anim. Sci.* 83(Suppl.):E22-E31.

Holbrook, T. C., T. Tripton, and D. McFarlane. 2012. Neutrophil and cytokine dysfunction in hyperinsulinemic obese horses. *Vet. Immunol. Immunopathol.* 145(1): 283-289.

Pagan J. D. 1998. Measuring the digestible energy content of horse feeds. *Advan. Equine Nutr.* 71-76. J. D. Pagan (ed). Nottingham, UK: Nottingham University Press.

Martin-Rossett, W. and M. Vermorel. 1991. Maintenance energy requirements determined by indirect calorimetry and feeding trials in light horses. *Equine Vet. Sci.* 11(1):42-45.

Jeffcott, L.B., J. R. Field, J. G. McLean, and K. Odea. 1986. Glucose-tolerance and insulin sensitivity in ponies and Standardbred horses. *Equine Vet. J.* 18(2): 97-101.

Rijnen, K. and J. H. Van Der Kolk. 2003. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies. *Am. J. Vet. Res.* 64(10): 1260-1264.

Jansen, W. L., J. V. D. Kuilen, S. N. J. Geelen, and A. C. Beynen. 2000. The effect of replacing nonstructural carbohydrates with soybean oil on the digestibility of fibre in trotting horses. *Equine Vet. J.* 32(1): 27-30.

Jansen, W. L., S. N. J. Geelen, J. Kuilen, and A. C. Beynen. 2002. Dietary soybean oil depresses the apparent digestibility of fibre in trotters when substituted for an iso-energetic amount of corn starch or glucose. *Equine Vet. J.* 34(3): 302-305.

Jansen, W. L., J. W. Cone, and S. N. J. Geelen, M. S. van Oldruitenborgh-Oosterbaan, A. H. Van Gelder, S. O. Elferink, and A. C. Beynen. 2007. High fat intake by ponies reduces both apparent digestibility of dietary cellulose and cellulose fermentation by faeces and isolated caecal and colonic contents. *Anim. Feed Sci. Technol.* 133(3): 298-308.

Edouard, N. G. Fleurance, W. Martin-Rosset, P. Duncan, J. P. Dulphy, S. Grange, R. Baumont, H. Dubroeuq, F. J. Perez-Barberia, and I. J. Gordon. 2008. Voluntary intake and digestibility in horses: Effect of forage quality with emphasis on individual variability. 1526-1533.

Senft, R. L., and L. R. Rittenhouse. 1985. A model of thermal acclimation in cattle. *J. Anim. Sci.* 61(2): 297-306.

Havel, P. J. 2001. Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. *Experim. Biol. Med.* 226(11): 963-977.

Ralston, S. L., D. E. Freeman, C. A. Baile. 1983. Volatile fatty acids and the role of the large intestine in the control of feed intake in ponies. *J. Anim. Sci.* 57(4): 815-825.

Stull, C. 2003. Nutrition for rehabilitating the starved horse. *J. Equine Vet. Sci.* 23(10): 456-457.

Stull, C. L., P. J. Hullinger, and A. V. Rodiek. 2003. Fat Supplementation to alfalfa diets for refeeding the starved horse. *Prof. Anim. Sci.* 19(1): 47-54.

Lindåse, S. S., K. E. Nostell, C. E. Müller, M. Jensen-Waern, and J. T. Bröjer. 2016. Effects of diet-induced weight gain and turnout to pasture on insulin sensitivity in moderately insulin resistant horses. *Amer. J. Vet. Res.* 77(3): 300-309.

Cymbaluk, N. F., and G. I. Christison. 1989. Effects of diet and climate on growing horses. *J. Anim. Sci.* 67(1): 48-59.

Bush, J. A., D. E. Freeman, K. H. Kline, N. R. Merchen, and G. C. Fahey, 2001. Dietary fat supplementation effects on in vitro nutrient disappearance and in vivo nutrient intake and total tract digestibility by horses. *J. Anim. Sci.* 79(1): 232-239.

Stillions, M. C., and W. E. Nelson. 1972. Digestible energy during maintenance of the light horse. *J. Anim. Sci.* 34(6): 981-982.

Geor, R. J., and P. Harris. 2009. Dietary management of obesity and insulin resistance: countering risk for laminitis. *Vet. Clin. North Amer. Equine Pract.* 25(1): 51-65.

- Gill, J. C., S. E. Pratt-Phillips R. Mansmann, and P. D. Siciliano. 2016. Weight loss management in client-owned horses. *J. Equine Vet. Sci.* 39:80-89
- Van Weyenberg, S., M. Hesta, J. Buyse, and G. P. J. Janssens. 2008. The effect of weight loss by energy restriction on metabolic profile and glucose tolerance in ponies. *Journal of animal physiology and animal nutrition.* *J. Anim. Physiol. Anim. Nutr.* 92(5): 538-545.
- Ungru, J., M. Blüher, M. Coenen, J. Raila, R. Boston, and I. Vervuert. 2012. Effects of body weight reduction on blood adipokines and subcutaneous adipose tissue adipokine mRNA expression profiles in obese ponies. *Vet. Record: J. Br. Vet. Assoc.* 171(21): 528.
- Rikke, B. A., M. E. Battaglia, D. B. Allison, and T. E. Johnson. 2006. Murine weight loss exhibits significant genetic variation during dietary restriction. *Physiol. Genom.* 27(2): 122-130.
- Bouchard, C., and A. Tremblay. 1997. Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. *J. Nutr.* 127(5): 943S-947S.
- Cooper, J., and P. McGreevy. 2007. Stereotypic behaviour in the stabled horse: causes, effects and prevention without compromising horse welfare. In *The welfare of horses.* Springer Netherlands. 99-124.
- Krzak, W. E., H. W. Gonyou, and L. M. Lawrence. 1991. Wood chewing by stabled horses: diurnal pattern and effects of exercise. *J. Anim. Sci.* 69(3): 1053-1058.
- Waters, A. J., C. J. Nicol, N. P. French. 2002. Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study. *Equine Vet. J.* 34(6): 572-579.
- Hothersall, B., and C. Nicol. 2009. Role of diet and feeding in normal and stereotypic behaviors in horses. *Vet. Clin. North Amer. Equine Pract.* 25(1): 167-181.
- Green, P., and J. M. Tong. 1988. Small intestinal obstruction associated with wood chewing in two horses. *Vet. Record* 123(8): 196-198.
- Curtis, G. C., C. F. Barfoot, A. H. Dugdale, P. A. Harris, and C. M. Argo, C. M. 2011. Voluntary ingestion of wood shavings by obese horses under dietary restriction. *Br. J. Nutr.* 106(S1): S178-S182.
- Reeves, M. J., M. D. Salman, and G. Smith. 1996. Risk factors for equine acute abdominal disease (colic): Results from a multi-center case-control study. *Prev. Vet. Med.* 26(3): 285-301.

- Siciliano, P. D., and S. Schmitt. 2012. Effect of restricted grazing on hindgut pH and fluid balance. *J. Equine Vet. Sci.* 32(9): 558-561.
- Stevens, V. J., E. Obarzanek, N. R. Cook, I-M. Lee, L. J. Appel, D. S. West, N. C. Milas, et al. 2001. Long-term weight loss and changes in blood pressure: results of the Trials of Hypertension Prevention, phase II. *An. Intern. Med.* 134(1): 1-11.
- Staessen, J., R. Fagard, and A. Amery. 1988. The relationship between body weight and blood pressure. *J. Hum. Hypertens.* 2(4): 207-217.
- Hollis, J. F., C. M. Gullion, V. J. Stevens, P. J. Brantley, L. J. Appel, J. D. Ard, Catherine M. Champagne, et al. 2008. Weight loss during the intensive intervention phase of the weight-loss maintenance trial. *Amer. J. Prev. Med.* 35(2): 118-126.
- Potter G. D., J. W. Evans, G. W. Webb, and S. P. Webb. 1987. Digestible energy requirements of Belgian and Percheron horses. *Proc. 10<sup>th</sup> Equine Nutri. Physiol. Soc. Symp.*, Ft. Collins, CO. 133-138.
- Heusner, G. 1993. Ad libitum feeding of mature horses to achieve rapid weight gain. *Proc. 13<sup>th</sup> Equine Nutr. Physiol. Soc. Symp.*, Gainesville, FL. 86.
- Muñoz, A., C. Riber, P. Trigo, and F. Castejón. 2010. Hematology and clinical pathology data in chronically starved horses. *J. Equine Vet. Sci.* 30(10): 581-589.
- Witham, C. L. and C. L. Stull. 1998. Metabolic responses of chronically starved horses to refeeding with three isoenergetic diets. *J. Am. Vet. Med. Assoc.* 212: 691-696.
- Berger, A., K. M. Scheibe, K. Eichhorn, A. Scheibe, and J. Streich. 1999. Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. *Appl. Anim. Behav. Sci.* 64(1): 1-17.
- Haupt, K. A. 2006. Mastication and feeding in horses. *Feeding in Domestic Vertebrates: From Structure to Behaviour.* 195.
- Kienzle, E., R. Bergler, and A. Mandernach. 1998. A comparison of the feeding behavior and the human-animal relationship in owners of normal and obese dogs. *J. Nutr.* 128(12): 2779S-2782S.
- Carciofi, A. C., K. N. V. Gonçalves, R. S. Vasconcellos, R. S. Bazolli, M. A. Brunetto, and F. Prada. 2005. A weight loss protocol and owners participation in the treatment of canine obesity. *Ciência Rural* 35(6): 1331-1338.

Yaissle, J. E., C. Holloway, and C. T. Buffington. 2004. Evaluation of owner education as a component of obesity treatment programs for dogs. *J. Amer. Vet. Med. Assoc.* 224(12): 1932-1935.

Bissot, T., E. Servet, S. Vidal, M. Deboise, R. Sergheraert, G. Egron, M. Hugonnard, S. E. Heath, V. Biourge, and A. J. German. 2010. Novel dietary strategies can improve the outcome of weight loss programmes in obese client-owned cats. *J. Feline Med. Surg.* 12(2): 104-112.

German, A. J., S. L. Holden, T. Bissot, R. M. Hackett, and V. Biourge. 2007. Dietary energy restriction and successful weight loss in obese client-owned dogs. *J. Vet. Intern. Med.* 21(6): 1174-1180.

Bott, R. C., E. A. Greene, K. Koch, K. L. Martinson, P. D. Siciliano, C. Williams, N. L. Trottier, A. Burk, and A. Swinker. 2013. Production and environmental implications of equine grazing. *J. Equine Vet. Sci.* 33(12): 1031-1043.

Hunt, W. F. 1995. Pastures for horses: a New Zealand perspective. *Proc. Equine Nutr. Pastur. Hor. Worksh.* Richmond, VA. 17-19.

Elphinstone, G. D. 1981. Pastures and fodder crops for horses in southern coastal Queensland. *Queensland Agric. J.*

Fleurance, G., P. Duncan, and B. Mallevaud. 2001. Daily intake and the selection of feeding sites by horses in heterogeneous wet grasslands. *Anim. Res.* 50(2): 149-156.

Hoskin, S. O., and E. K. Gee. 2004. Feeding value of pastures for horses. *New Zealand Vet. J.* 52(6): 332-341.

Gallagher, J. R., and N. P. McMeniman. 1988. The nutritional status of pregnant and nonpregnant mares grazing South East Queensland pastures. *Equine Vet. J.* 20(6): 414-416.

Crowell-Davis, S. L., K. A. Houpt, and J. Carnevale. 1985. Feeding and drinking behavior of mares and foals with free. *J. Anim. Sci.* 60(4): 883-889.

Chavez, S. J., P. D. Siciliano, G. B. Huntington. 2014. Intake estimation of horses grazing tall fescue or fed tall fescue hay. *J. Anim. Sci.* 92(5): 2304-2308.

Ince, J., A. C. Longland, J. C. Newbold, P. A. Harris. 2011. Changes in proportions of dry matter intakes by ponies with access to pasture and haylage for 3 and 20 hours per day respectively, for six weeks. *J. Equine Vet. Sci.* 31(5): 283.

Grace, N. D., E. K. Gee, E. C. Firth, and H. L. Shaw. 2002. Digestible energy intake, dry matter digestibility and mineral status of grazing New Zealand Thoroughbred yearlings. *New Zealand Vet. J.* 50(2): 63-69.

Rayburn, E., and J. Lozier. 2003. A falling plate meter for estimating pasture forage mass. WVU Extension Service e-factsheet.

Rayburn, E. B., and S. B. Rayburn. 1998. A standardized plate meter for estimating pasture mass in on-farm research trials. *Agron. J.* 90(2): 238-241.

Cantillon, J. D. 1986. Measurement of intake levels and examination of grazing parameters of Quarter Horse geldings.

Haenlein, G. F. W., R. C. Smith, and Y. M. Yoon. 1966. Determination of the fecal excretion rate of horses with chromic oxide. *J. Anim. Sci.* 25: 1091-1095.

Bergero, D., C. Préfontaine, N. Miraglia, and P. G. Peiretti. 2009. A comparison between the 2N and 4N HCl acid-insoluble ash methods for digestibility trials in horses. *Anim.* 3(12): 1728-1732.

Bergero, D., N. Miraglia, C. Abba, and M. Polidori. 2004. Apparent digestibility of Mediterranean forages determined by total collection of feces and acid-insoluble ash as internal marker. *Livest. Prod. Sci.* 85(2): 235-238.

Cuddeford, D., and D. Hughes. 1990. A comparison between chromium-mordanted hay and acid-insoluble ash to determine apparent digestibility of a chaffed, molassed hay/straw mixture. *Equine Vet. J.* 22(2): 122-125.

Miraglia, N., D. Bergero, B. Bassano, M. Tarantola, and G. Ladetto. 1999. Studies of apparent digestibility in horses and the use of internal markers. *Livest. Prod. Sci.* 60(1): 21-25.

Sutton, E. I., J. P. Bowland, and J. F. McCarthy. 1977. Studies with horses comparing 4N-HCl insoluble ash as an index material with total fecal collection in the determination of apparent digestibilities. *Can. J. Anim. Sci.* 57(3): 543-549.

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44(2): 282-287.

Dove, H., R. W. Mayes, and M. Freer. 1996. Effects of species, plant part, and plant age on the n-alkane concentrations in the cuticular wax of pasture plants. *Crop Past. Sci.* 47(8): 1333-1347.

Stevens, D. M., J. B. J. Van Ryssen, and J. P. Marais. 2002. The use of n-alkane markers to estimate the intake and apparent digestibility of ryegrass and Kikuyu by horses. *S. African J. Anim. Sci.* 32(1): 50-56.

Menard, C., P. Duncan, G. Fleurance, J. Y. Georges, and M. Lila. 2002. Comparative foraging and nutrition of horses and cattle in European wetlands. *J. Appl. Ecol.* 39(1): 120-133.

Shrivastava, V. S., and S. K. Talapatra. 1962. Pasture studies in Uttar Pradesh. 3. Determination of pasture consumption by grazing animal. *Ind. J. Dairy Sci.* 15: 161-166.

Dulphy, J. P., W. Martin-Rosset, H. Dubroeuq, J. M. Ballet, A. Detour, and M. Jailler. 1997. Compared feeding patterns in ad libitum intake of dry forages by horses and sheep. *Livest. Prod. Sci.* 52(1): 49-56.

Chenost, M., and W. Martin-Rosset. 1985. Comparison between species (sheep, horses, cattle) on intake and digestibility of fresh herbage. *Ann. Zootech.* 34: 291-312.

Marlow, C. H., E. M. Van Tonder, F. C. Hayward, S. S. Van der Merwe, and L. E. Price. 1983. A report on the consumption, composition and nutritional adequacy of a mixture of lush green perennial ryegrass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*) fed ad libitum to Thoroughbred mares. *J. S. African Vet. Assoc.* 54(3): 155-157.

Glunk, E. C., S. E. Pratt-Phillips, and P. D. Siciliano. 2013. Effect of restricted pasture access on pasture dry matter intake rate, dietary energy intake, and fecal pH in horses. *J. Equine Vet. Sci.* 33(6): 421-426.

Longland, A. C., and B. M. Byrd. 2006. Pasture nonstructural carbohydrates and equine laminitis. *J. Nutr.* 136(7): 2099S-2102S.

Duren, S. E., C. T. Dougherty, S. G. Jackson, and J. P. Baker. 1989. Modification of ingestive behavior due to exercise in yearling horses grazing orchardgrass. *Appl. Anim. Behav. Sci.* 22(3-4): 335-345.

Collas, C., B. Dumont, R. Delagarde, W. Martin-Rosset, and G. Fleurance. 2015. Energy supplementation and herbage allowance effects on daily intake in lactating mares. *J. Anim. Sci.* 93(5): 2520-2529.

Holland, J. L., D. S. Kronfeld, D. Sklan, and P. A. Harris. 1998. Calculation of fecal kinetics in horses fed hay or hay and concentrate. *J. Anim. Sci.* 76(7): 1937-1944.

Hudson, J. M., N. D. Cohen, P. G. Gibbs, J. A. Thompson. 2001. Feeding practices associated with colic in horses. *J. Amer. Vet. Med. Assoc.* 219(10): 1419-1425.

- Siciliano, P. D. 2012. Estimation of pasture dry matter intake and its practical application in grazing management for horses. Proc. 10th Mid-Atlantic Nutr. Conf., Timonium, MD, 9-12.
- Dowler, L. E., P. D. Siciliano, S. E. Pratt-Phillips, M. Poore. 2012. Determination of pasture dry matter intake rates in different seasons and their application in grazing management. J. Equine Vet. Sci. 32(2): 85-92.
- Ince, J., A. C. Longland, J. C. Newbold, and P. A. Harris. 2011. Changes in proportions of dry matter intakes by ponies with access to pasture and haylage for 3 and 20 hours per day respectively, for six weeks. J. Equine Vet. Sci. 31(5): 283.
- Longland, A. C., C. Barfoot, P. A. Harris. 2011. The effect of wearing a grazing muzzle vs not wearing a grazing muzzle on pasture dry matter intake by ponies. J. Equine Vet. Sci. 31(5): 282-283.
- Glunk, E. C., C. C. Sheaffer, M. R. Hathaway, and K. L. Martinson. 2014. Interaction of grazing muzzle use and grass species on forage intake of horses. J. Equine Vet. Sci. 34(7): 930-933.
- Glunk, E. C., C. C. Sheaffer, M. R. Hathaway, and K. L. Martinson. 2013. The interaction of grazing muzzle use and grass species on forage intake of horses: a preliminary study. J. Equine Vet. Sci. 33(5): 357.
- McIntosh, B., D. Kronfeld, R. Geor, W. Stanier, A. Longland, L. Gay, D. Ward, and P. Harris. 2007. Circadian and seasonal fluctuations of glucose and insulin concentrations in grazing horses. Proc. 20th Equine Sci. Soc. 100-101.
- Chatterton, N. J., P. A. Harrison, J. H. Bennett, and K. H. Asay. 1989. Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. J. Plant Physiol. 134(2): 169-179.
- Kagan, I. A., B. H. Kirch, C. D. Thatcher, J. R. Strickland, C. D. Teutsch, F. Elvinger, and R. S. Pleasant. 2011. Seasonal and diurnal variation in simple sugar and fructan composition of orchardgrass pasture and hay in the Piedmont region of the United States. J. Equine Vet. Sci. 31(8): 488-497.
- Wycoff, C. L., E. C. Glunk, C. C. Sykes, S. Rodpitak, S. E. Pratt-Phillips, and P. D. Siciliano. 2013. Effect of day-time vs. night-time grazing on hindgut fermentation parameters in fall pasture-fed horses. J. Equine Vet. Sci. 33(5): 338-339.
- Siciliano, P. D., and J. C. Gill. 2015. Effect of pasture sward height on blood glucose and insulin profiles in grazing horses. J. Equine Vet. Sci. 35(5): 429.

- Lewis, L. D. 1995. Equine clinical nutrition: feeding and care. Williams & Wilkins.
- Houpt, K. A. 1990. Ingestive behavior. *The Veterinary Clinics of North America. Equine Pract.* 6(2): 319-337.
- Shingu, Y., M. Kawai, H. Inaba, S. Kondo, H. Hata, and M. Okubo. 2000. Voluntary intake and behavior of Hokkaido native horses and light half-bred horses in woodland pasture. *J. Equine Sci.* 11(3): 69-73.
- Arnold, G. W. 1984. Comparison of the time budgets and circadian patterns of maintenance activities in sheep, cattle and horses grouped together. *Appl. Anim. Behav. Sci.* 13(1-2): 19-30.
- Rivera, E., S. Benjamin, B. Nielsen, J. Shelle, and A. J. Zanella. 2002. Behavioral and physiological responses of horses to initial training: the comparison between pastured versus stalled horses. *Appl. Anim. Behav. Sci.* 78(2): 235-252.
- Bell, R. A., B. D. Nielsen, K. Waite, D. Rosenstein, and M. Orth. 2001. Daily access to pasture turnout prevents loss of mineral in the third metacarpus of Arabian weanlings. *J. Anim. Sci.* 79(5): 1142-1150.
- Graham-Thiers, P. M., and L. K. Bowen. 2013. Improved ability to maintain fitness in horses during large pasture turnout. *J. Equine Vet. Sci.* 33(8): 581-585.
- Hampson, B. A., J. M. Morton, P. C. Mills, M. G. Trotter, D. W. Lamb, and C. C. Pollitt. 2010. Monitoring distances travelled by horses using GPS tracking collars. *Austr. Vet. J.* 88(5): 176-181.
- Eaton, M. D., D. R. Hodgson, D. L. Evans, W. L. Bryden, and R. J. Rose. 1995. Effect of a diet containing supplementary fat on the capacity for high intensity exercise. *Equine Vet. J.* 27(S18): 353-356.
- McCutcheon, L. J., R. J. Geor, and K. W. Hinchcliff. 1999. Effects of prior exercise on muscle metabolism during sprint exercise in horses. *J. Appl. Physiol.* 87(5): 1914-1922.
- Tyler, C. M., D. R. Hodgson, and R. J. Rose. 1996. Effect of a warm-up on energy supply during high intensity exercise in horses. *Equine Vet. J.* 28(2): 117-120.
- Eaton, M. D., D. R. Hodgson, D. L. Evans, and R. J. Rose. 1999. Effects of low-and moderate-intensity training on metabolic responses to exercise in Thoroughbreds. *Equine Vet. J.* 31(S30): 521-527.
- Coenen, M. 2005. About the predictability of oxygen consumption and energy expenditure in the exercising horse. *Proc. 19th Equine Sci. Soc., Tuscon, AZ*

Hiraga, A., M. Kai, K. Kubo, K., Y. Yamaya, and B. K. Erickson. 1995. The effects of incline on cardiopulmonary function during exercise in the horse. *J. Equine Sci.* 6(2): 55-60.

Kingston, J. K., G. M. Soppet, C. W. Rogers, and E. C. Firth. 2006. Use of a global positioning and heart rate monitoring system to assess training load in a group of thoroughbred racehorses. *Equine Vet. J.* 38(S36): 106-109.

Yamamoto, S. 1989. Estimation of heat production from heart rate measurement of free living farm animals. *JARQ* 23: 134-143.

Booth, M. E. 1998. Factors influencing the energy requirements of native ponies living outdoors in the United Kingdom. Doctoral dissertation, University of Edinburgh.

Rogalski, M. 1974. Comparison of some characteristic interdependent factors between the sward and the grazing animals. Sectional Papers International Grassland Congress.

Rogalski, M. 1984. Preference for some types of grasses and intake of pasture by English Thoroughbred mares. *Herbage Abstracts*. 056-07146.

Moffitt, D. L. 1987. Seasonal differences in apparent digestibilities of fescue and orchardgrass/clover pastures by horses. Doctoral dissertation, Virginia Polytechnic Institute and State University.

Martin, R. G. 1993. Effects of nitrogen and energy supplementation on grazing broodmares and aspects of nitrogen balance in horses. Ph. D. Thesis. University of Queensland, Brisbane.

Dove, H. 2010. Keynote: Assessment of intake and diet composition of grazing livestock. *Proc. Graz. Livest. Nutr. Conf.* July 9 and 10, 2010. Australia.

Murray, M. J. 1994. Gastric ulcers in adult horses. The compendium on continuing education for the practicing veterinarian (USA).

Derksen, F. J., N. E. Robinson, P. J. Armstrong, J. A. Stick, and R. F. Slocombe. 1985. Airway reactivity in ponies with recurrent airway obstruction (heaves). *J. Appl. Physiol.* 58(2): 598-604.

## CHAPTER II. EXPERIMENT 1

### Weight loss management in client-owned horses

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#### 2.1 Abstract

The present study was designed to determine whether a reduction in body weight (BW) and body condition score (BCS) could be achieved through 10% to 20% energy restriction in privately-owned and managed horses, similar to previously published research in a controlled research setting. Twenty-four, overweight, client-owned/managed horses were identified for use in the study. At the initial (September 2012) and final evaluations (May 2013), health records, diet evaluation, estimated BW, fasting glucose and insulin (INS) concentrations, BCS, cresty neck score (CNS), and morphometric measurements were obtained from 24 client-owned horses and four control horses. At the conclusion of the trial period ( $26 \pm 4$  weeks), changes in BW, BCS, CNS, morphometric measurement and INS were analyzed using paired *t* tests. Correlations between change in BW (%initial) and  $\Delta$ BCS were evaluated. Overall decreases ( $p < 0.01$ ) were observed in BW (31.48 kg; 95% CI: 24.07 to 38.89 kg), BCS (1.4/9; 95% CI: 1.01 to 1.80), CNS (0.96; 95% CI: 0.64 to 1.30), and INS (9.132  $\mu$ U/ml; 95% CI: 5.741 to 12.520). Change in BW (%initial) and  $\Delta$ BCS were moderately correlated ( $r = 0.53$ ). Horses with more compliant owners had a greater mean decrease in BW and BCS ( $p < 0.01$ ) than those of non-compliant owners. Likewise, horses with no pasture access had a greater mean decrease in BW and BCS ( $p < 0.01$ ) compared to continuously grazing horses. Results suggest that 10% to 20% restriction of energy

intake can be implemented in client-owned horses, resulting in a decrease in BW, BCS, and INS, if the owner reduces feed and pasture intake.

## 2.2 Introduction

The results of several studies suggest that a large percentage of the world's horse population is overweight or obese. It is estimated that between 40% to 50% of horses are a body condition score (BCS)  $\geq 6/9$ , with 18.7% to 20% meeting obesity standards (BCS  $> 7/9$ ) (Dugdale et al. 2010, Harker et al. 2011, Pratt-Phillips et al. 2010, Thatcher et al. 2012, Wyse et al. 2011). Overweight horses may have an increased risk of laminitis, insulin (INS) resistance, and equine metabolic syndrome (Frank et al. 2010, Geor 2008, 2010, Gossellin et al. 2007, Hoffman et al. 2003, Suagee et al. 2013, Ungru et al. 2012). Obesity has been hypothesized to contribute to INS resistance through the disruption of INS signaling pathways by adipokines and pro-inflammatory cytokines (Bailey and Bamford 2013, Holbrook et al. 2012, Piya et al. 2013, Vick et al. 2007, McCue et al 2015). Several studies have linked the INS-resistant phenotype to a predisposition for laminitis (Johnson et al. 2009, Treiber et al. 2006). Furthermore, it has been reported that the risk for laminitis is the highest when horses are grazing lush pasture, particularly in the spring and late fall (Geor 2010, King and Mansmann 2004). The large number of obese horses and the risks associated with obesity has necessitated research investigating methods for weight loss in horses.

A reduction of body weight (BW), body condition, and basal INS have been achieved by restricting calorie and dry matter intake in controlled conditions over 6 to 18 week duration of study. Feeding 1.0% to 1.25% BW in dry matter intake (DMI) and

67% to 80% of NRC (2007) maintenance digestible energy (DE) requirements per day resulted in between 6.8% to 18.2% BW reduction and a BCS decrease of  $0.3 \pm 0.14$  to 4.0 units (BCS on a scale of 1-9) (Argo et al. 2012, Dugdale et al. 2010, McGowan et al. 2013, NRC 2007, Van Weyenberg et al. 2008). Several of these studies suggest even greater restriction in digestible energy intake is necessary in some horses that are more resistant to weight loss (Argo et al. 2012, Dugdale et al. 2010, Van Weyenberg et al. 2008). Weight reduction has resulted in a significant decrease in basal insulin concentration in horses and ponies (Argo et al. 2012, McGowan et al. 2013, Van Weyenberg et al. 2008).

Although the reversal of obesity can be achieved in a controlled research setting, little research in the equine field is available to confirm that dietary restriction practices can be implemented by horse owners and barn managers resulting in similar weight loss results and subsequent health benefits. To achieve the health benefits associated with adiposity reduction, horse owners and managers must be able to successfully apply a weight loss program. However, studies in dogs have found that it was common for weight loss to be unsuccessful due to a lack of owner compliance with the feeding protocols (Bouchard and Tremblay 1997, Gentry 2006, Gosselin et al. 2007, Laflamme and Kuhlman 1995, Lund et al. 2006, Wang and Lui 2008). German and colleagues (2007) found that clinicians should expect slower results in weight loss for client-owned dogs in comparison to those in a research setting because of differences in exercise levels, ambient temperature, concurrent illness, and owner compliance with the dietary regimen.

The level of diet restriction is important for weight loss; however, diets fed to induce rapid weight loss may predispose a horse to digestive or behavioral complications (Argo et al. 2012). Therefore, modest weight reduction methods over longer durations are considered to be more practical methods for animal owners. The purpose of this study was to determine the effectiveness of 10% to 20% calorie restriction for weight loss in overweight horses that were managed by their owners at privately owned horse stables.

## 2.3 Materials and Methods

The following experimental protocol was approved by the North Carolina State University Animal Care and Use Committee.

### *2.3 a. Animals and Physical Evaluation*

This study took place at 17 privately owned horse stables within a 100-mile radius of Raleigh, North Carolina from September 2012 to May 2013. In September, owners willing to participate their horses in the weight loss study were identified through various multimedia websites or were referred to us by their veterinarians. Twenty-four, client-owned horses, consisting of 12 geldings and 12 mares (mean  $\pm$  standard error;  $530 \pm 15$  kg BW;  $11.6 \pm 0.7$  years; mixed breeds) qualified for use in the study (BCS  $\geq 6.5/9$ ; Table 4). Initial consultations with horse owners were scheduled from September to November, and final consultations were scheduled from April to May. Therefore, the weight loss trial spanned  $26 \pm 4$  weeks. Most horses were clinically normal at the start of the study; however some reported previous medical conditions: Two horses were diagnosed with acute laminitis prior to the start of the study and were

undergoing treatment; four horses had been previously diagnosed with laminitis but were clinically normal at the start of the study; one horse had white line disease and was undergoing treatment; one young horse had a deformation of the left proximal phalanx; and one horse was reported to have exercise intolerance by the owner. All horses with preexisting medical conditions were being actively treated by a veterinarian. Horses recently diagnosed with acute laminitis were beginning to implement dietary changes, although no weight loss was achieved from the start of veterinary treatment. At the beginning of the study, horse name, age, breed, and sex were logged, and the initial and final parameters that were evaluated included, morphometric measurements as described by Carter and colleagues (2009); heart girth (HG), neck crest (NC), length, and height, BCS, cresty neck score (CNS), exercise routine (hours of exercise per week, intensity of work, discipline), and the current management practices. Body condition scores were evaluated on a scale of 1 to 9 using the scoring system devised by Henneke and colleagues (1983). Body length was measured from the point of the shoulder to the point of the buttock (ischiatric tuberosity), and girth circumference was measured caudal to the elbow over the highest point of the withers (Carter et al. 2009). Body weight (BW) was estimated using the formula:  $BW (kg) = [girth (cm)^2 \times length (cm)] / 11,877$  (Carroll and Huntingdon 1988). Neck circumference was taken midway between the poll and withers (Carter et al. 2009).

Four geldings, selected from the North Carolina State University research herd (mean  $\pm$  standard error;  $500 \pm 56$  kg BW; 6-12 years; Quarter Horses), were used in the study as controls that were not subject to caloric restriction. Morphometric measurements (HG, NC, length, and height), BCS, CNS, estimated weight, and scale

weight were obtained at the beginning (October 11, 2012) and end (May 2, 2013) of the trial period. These horses were maintained on pasture continuously and offered grass hay free choice when pasture was not available in the winter months.

### *2.3 b. Dietary Protocols*

A comprehensive nutritional evaluation was completed for each horse at the initial and final consultations. The initial body weight and physiological status of each horse was used in order to generate their initial nutrient requirement, based on the NRC (2007). Initially, daily offered amounts of hay and concentrate were weighed and recorded, and pasture intake was estimated at 2.0% BW in DMI per day (NRC 2007). Hay and pasture were sampled and evaluated by a laboratory for chemical content by the North Carolina Department of Agriculture and Consumer Services feed profiling laboratory (Raleigh, NC). The digestible energy content of hay and pasture was calculated using the equation published by Pagan:  $DE \text{ (kcal/kg DM)} = 2,118 + 12.18 \text{ (CP\%)} - 9.37 \text{ (ADF \%)} - 3.83 \text{ (hemicellulose\%)} + 47.18 \text{ (fat\%)} + 20.35 \text{ (NSC\%)} - 26.3 \text{ (ash\%)}$  (Pagan 1998). The DE content was multiplied by the %DM of the forage to yield the DE content on an “as-fed” basis. The DE content of concentrates and supplements was provided by the feed label, the manufacturer, or the equation,  $3.25812 + 0.0522 \text{ (fat\%)} - 0.03417 \text{ (fiber\%)}$ . Then, the DE content (Mcal) of the feed was multiplied by the daily amount offered (kg) to calculate a total DE energy intake (Mcal/kg).

New rations were then formulated to meet 80 to 90% of maintenance DE requirements and to provide between 1.25% to 1.50% of BW in DMI to facilitate weight loss and a decrease in BCS. Owners fed a vitamin and mineral supplement (SmartVite, SmartPak Equine LLC., or Micro-Phase, Kentucky Performance Products LLC., Versailles,

KY, chosen based on the best match to the nutrient requirements, availability, and palatability) to provide a balanced ration when feed intake was reduced. Any changes to the diet, i.e., different hay, pasture, or grain during the trial period were re-evaluated.

Pasture was evaluated based on a subjective scale of lush, limited, sparse, or dry lot. Based on the timing of the initial visit, pasture availability was generally lush for the majority of the facilities. Because approximately 80% of horses had unrestricted access to pasture at the beginning of the study, several methods were used for restricting calorie intake in horses, which involved either removing horses from pasture (dry lot), restricting hours at pasture, or through the use of a grazing muzzle (Best Friend Equine Supply, New Holland, PA) that were supplied by the researchers. The suggested method for reducing pasture intake depended on the individual facility accommodations. Three horses had already been moved to dry lot turnout based on veterinarian recommendations approximately two weeks before the start of the study. When a grazing muzzle was recommended, a reduced pasture intake was calculated using Longland's equation, which was modified for hourly intake ( $0.0453\% \text{ BW (kg)/hr}$ ) (Longland et al. 2012). When time at pasture was restricted, pasture consumption per hour was calculated using the DMI rate published by Dowler ( $0.121 \text{ kg DM/100 kg BW/hour at pasture}$ ) (Dowler et al. 2012). This equation estimates the DMI rate for horses grazing 8 h/d in different seasons of the year.

The new dietary protocols were assigned to each owner and horse pairing via email. Owners were provided graphs demonstrating how the prescribed diet compared with the horse's nutrient and energy requirement (NRC 2007). Only digestible energy and dry matter intake were restricted; protein, vitamins, and minerals were maintained

at or above requirement level (NRC 2007). Written instructions that were sent to owners included a complete feeding protocol containing the exact weight amount of hay (or by flake weight) and concentrate to feed, methods for reducing or eliminating pasture (i.e., grazing muzzles were sent to owners), type and brand of ration balancer to purchase, and management instructions to follow. Owners we asked to feed this diet for the duration of the study. Any modifications to the protocol were to be addressed through email or phone conversations, and in some cases, the researchers revisited facilities to weigh and sample new rations. Owners were asked to weigh rations in order to feed the proper amount, although no written record was required by the researchers because of practical limitations. Owners were provided with a weight tape to track their horse's progress.

### *2.3 c. Final Consultation*

Twenty-three out of the twenty-four horses were re-evaluated at the end of the trial period (one horse relocated), and changes in BW and BCS were determined. Horses were then categorized into groups based on the three types of management situations that were ultimately implemented by the owners: horses with continuous pasture access (24 hours; n=13), horses with partial access to pasture (time-restricted or grazing muzzle-restricted) (n=4), and horses with no access to pasture (dry lot or stall confined) (n=6). Horses with continuous pasture were grazing/had access to pasture for 24 hours per day. Horses with partial pasture were turned out between 8 and 14 hours per day and spent the remaining time in a dry lot, stall or were limited in pasture intake using a grazing muzzle. Horses with no pasture access were confined to a dry lot or stall for 24 hours per day.

A record of the compliance of horse owners/managers with the dietary protocols was maintained throughout the duration of the study by the researchers. Owners were asked to weigh rations, use grazing muzzles, create dry lots, reduce feed intake, as well as stay in communication with the researchers in regards to modifications to or complications with the protocol. Owner compliance was evaluated and owners were rated in accordance to their willingness and ability to apply the weight loss protocols based on a 1- to 3-point scale (1=little to no compliance, 2=some compliance, 3=good compliance). Owners who scored a 1 in compliance (n=6) did not follow the dietary protocol or may have minimally reduced feeding of hay/and or concentrates, and did not make any attempt to restrict pasture consumption. Owners who scored a 2 in compliance (n=10) were inconsistent in their ability to carry out the protocols; however, they were able to reduce hay, concentrate, and/or pasture consumption in their horses. Owners who scored a 3 in compliance (n=7) were able to restrict hay, concentrate, and/or pasture intake to the desired intake level. Owners who scored a 3 in compliance responded to emails or kept in email communication with researcher, reported weighing rations, and generally expressed concern for improving their horse's health. It should be noted that there was some overlap with owner compliance level 1 and the unrestricted pasture group; however, some owners of horses with unrestricted pasture access that were able to consistently restrict their horse's intake in other ways (i.e., concentrate or hay) received a compliance score of >1.

#### *2.3 d. Blood Sampling and Analysis*

Blood samples were collected from 23 of the 24 horses at the initial consultation (one horse was unavailable for sampling) and 22 of the 24 horses at the final

consultation (two horses were unavailable for sampling, including one horse that had relocated) via jugular venipuncture using an 18-G needle and two Vacutainer tubes (one containing ethylenediamine tetracetic acid dipotassium salt (EDTA) and one containing no-additive). Blood samples were collected 6 to 12 hours after feed was withheld. Samples were processed for whole blood glucose (GLU) and serum insulin analysis. Blood GLU was analyzed using whole blood immediately after sample collection using a glucose monitor (ReliOn Ultima, Abbott Diabetes Care, Inc., Alameda, CA, USA). Samples were chilled on ice, followed by centrifugation (10,000 rpm for 10 min) for the separation of plasma and serum within 2 h of collection. The plasma and serum samples were stored at -20 °C until further analysis. Serum INS was analyzed in duplicates using a commercially available RIA kit (Coat-A-Count, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA), previously validated for horse blood (Reimers et al. 1982). Standards were diluted to account for low resting insulin concentrations and the lowest detectable limit of the assays were either 1.01 or 1.09  $\mu\text{U/mL}$ . The intraassay and interassay coefficient of variations were 4.8% and 7.7%, respectively.

Blood GLU and INS concentrations were analyzed from the control horses following similar procedures at the beginning and end of the trial period. Control horses were brought in overnight and were therefore off feed for approximately 12 hour before sampling.

### *2.3 e. Statistical Analysis*

Differences in the initial and final within-horse measures for BW, BCS, CNS, NC, HG, GLU, and INS were analyzed using paired *t* tests (Motulsky 2007). All results for the paired *t* tests were presented as the mean difference with the associated 95% CI.

Correlation between horse parameters of  $\Delta$ BW (%),  $\Delta$ BCS, and  $\Delta$ INS were calculated using Pearson's correlation coefficients (Walker 2002). Horses were grouped according to pasture access (e.g., no pasture, partial pasture, and continuous pasture). Change in BW (%) and BCS among pasture access groups were analyzed using analysis of variance (ANOVA) for completely randomized designs, and the means were separated using the Tukey-Kramer method of pairwise comparison if the overall ANOVA model was significant (Walker 2002). In a separate model, horses were grouped according to owner compliance level (e.g., in increasing compliance, 1, 2, or 3). Change in BW (%) and BCS among owner compliance groups were analyzed using ANOVA for completely randomized designs and means were separated using the Tukey-Kramer method of pairwise comparison if the overall ANOVA model was significant (Walker 2002). Results for the pairwise comparisons are represented as mean  $\pm$  standard error of the mean. Significance was accepted at  $p < 0.05$ .

## 2.4 Results

### 2.4 a. Overall Change in Body Composition

Overall, the BW (kg) and BCS of the horses were significantly lower ( $P < .001$ ) at the end of the trial than at the beginning, as shown in Table 5. This represented a mean  $\Delta$ BW of -5.98% (95% CI: -4.56 to -7.41) and a mean  $\Delta$ BCS of -1.4/9 (95% CI: -1.0 to -1.8). Change in BW (%) and  $\Delta$ BCS were moderately correlated ( $r=0.53$ ;  $P < .01$ ; Fig. 2). Mean within horse CNS (-1.0; 95% CI: -0.6 to -1.3), NC (-5.28 cm; 95% CI: -2.69 to -7.90), and HG (-4.88 cm; 95% CI: 1.11 to -9.35) measurements at the conclusion of the trial were significantly reduced ( $p < 0.01$ ) from beginning values.

The mean  $\Delta BW$  (%) and  $\Delta BCS$  were compared according to pasture group. The mean  $\Delta BW$  (%) and  $\Delta BCS$  for horses in the continuous pasture intake group (n=13) were  $-4.38 \pm 0.68\%$  and  $-1.0 \pm 0.2$ , respectively. The mean  $\Delta BW$  (%) and  $\Delta BCS$  for horses in the partial pasture intake group (n=4) were  $-7.20 \pm 1.16\%$  and  $-1.5 \pm 0.3$ , respectively. The mean  $\Delta BW$  (%) and  $\Delta BCS$  for the no pasture intake group (n=6) were  $-9.80 \pm 1.55\%$  BW and  $-2.3 \pm 0.4$ , respectively. The pairwise comparison analysis (Tukey-Kramer) for the difference in the means for the effect of pasture intake on BW (%) yielded significantly greater ( $p < 0.01$ ) weight loss for the no pasture group than the continuous pasture group; however, no significant difference ( $p = 0.76$ ;  $p = 0.107$ ) in BW (%) was observed between the no pasture and partial pasture group means, or the partial pasture and the continuous pasture group means (Fig. 3a). The pairwise comparison analysis (Tukey-Kramer) for the difference in the means for the effect of pasture intake on BCS yielded significantly greater ( $p = .005$ ) BCS decrease for the no pasture than the continuous pasture group means; however, no significant difference ( $p = 0.21$ ;  $p = 0.50$ ) was observed between the no pasture and the partial pasture group means, or the partial pasture and the continuous pasture group means (Fig. 3b).

For the control horses, initial and final within horse mean BW (kg), BCS, CNS, NC, and HG is shown in Table 3. Control horses exhibited an increase in BW (kg) by 35.25 kg (95% CI: -6.87 to 77.37) and BCS by 0.7 (95% CI: 0.1 to 1.3). Mean within horse HG and NC were significantly higher at the end of the trial period (Table 6).

#### *2.4 b. Dietary Intake Analysis*

The initial dietary evaluations yielded a mean estimated digestible energy intake (DEI) of  $45.2 \pm 1.6$  Kcal/kg BW, which was substantially greater than the NRC

maintenance DE requirement for mature, idle horses of 33.3 Kcal/kg BW (NRC 2007).

The dietary protocols that were given to owners to follow for the trial period resulted in a mean of  $28.5 \pm 1.0$  Kcal/kg in energy intake ( $85.4 \pm 1.5\%$  NRC DE requirement based on maintenance or minimum maintenance requirements) and  $1.32 \pm 0.04\%$  in DMI, while maintaining protein, vitamins, and minerals at the requirement level (Table 7).

#### *2.4 c. Owner Compliance*

The mean  $\Delta$ BWs (%) for the owner compliance levels 1, 2, and 3 were  $-2.4 \pm 0.5\%$  (n=6),  $-6.2 \pm 0.7\%$  (n=10), and  $-8.8 \pm 1.4\%$  (n=7), respectively. The pairwise comparison analysis (Tukey-Kramer) for the difference in the means for the effect of owner compliance level on  $\Delta$ BW (%) was significant for owner compliance levels 1 and 3 ( $p < 0.01$ ) and 1 and 2 ( $p = 0.026$ ), however, levels 2 and 3 were not significantly different in respect to  $\Delta$ BW (%;  $p = 0.146$ ; Fig 4a). Mean  $\Delta$ BCS for owner compliance levels 1, 2, and 3 were  $-0.8 \pm 0.3$ ,  $-1.1 \pm 0.2$ , and  $-2.3 \pm 0.2$ , respectively. The pairwise comparison analysis of the means for the effect of owner compliance on  $\Delta$ BCS was significantly different between levels 1 and 3 ( $p < 0.01$ ) and 2 and 3 ( $p < 0.01$ ), however, mean  $\Delta$ BW (%) was not significantly different between levels 1 and 2 ( $p = 0.585$ ; Fig. 4b).

#### *2.4 d. Insulin and Glucose Concentrations*

Baseline GLU was not affected by the amount of weight loss, and remained relatively unchanged overall ( $-2.90$  mg/dL; 95% CI:  $-13.74$  to  $7.94$ ;  $p = 0.58$ ; Table 5). There was an overall decrease in INS by  $9.132$   $\mu$ U/mL (95% CI:  $5.741$  to  $12.520$ ) ( $p < 0.01$ ), and there were no significant correlations of either  $\Delta$ BW or  $\Delta$ BCS with  $\Delta$ INS

( $p=0.53$ ;  $p=0.85$ ). For the control horses, final INS significantly increased by 17.65  $\mu\text{U/mL}$  (95% CI: 11.46 to 23.84) ( $p<0.01$ ).

## 2.5 Discussion

Dietary restriction of client-owned and managed overweight horses to approximately 85% of digestible energy maintenance requirements and 1.32% of DMI for 26 weeks resulted in approximately 6% BW reduction and a 1.4/9-unit decrease in BCS. The application of owner-conducted weight loss protocols in this field study resulted in the successful reduction of adiposity in the horses and equipped horse owners with nutritional awareness for managing obesity. Studies by Dugdale et al. (2010), Argo et al. (2012), McGowan et al. (2013), and Van Weyenberg et al. (2007) reported feeding 22.32, 27.34, 21.30, and 17.54 kcal/kg BW in DEI, respectively, and horses lost 11.4%, 7.4%, 6.8%, and 18.2% of initial body weight, respectively, over varying durations of study. A regression of calories fed (kcal/kg BW) versus mean weight loss (%BW) shows that, in comparison with the dietary intake level proposed in this study (28.50 Kcal/kg BW), the mean weight loss of the current study was acceptable. Based on a regression equation that was generated using the digestible energy intake and weight reduction data from previous studies,  $y = 0.987x + 32.784$  ( $R^2=0.576$ ), the predicted weight loss for the current study would have been 4.65% BW. Studies in client-owned dogs and cats found that slower rates of weight loss are to be expected when compared to an experimental setting, which may result from practical limitations, such as less-intensive supervision, varying degrees of activity level, owner noncompliance, and differences in onset variables, such as age, sex, and neuter status

(Bissot et al. 2010, German et al. 2007). Higher levels of weight loss could have been achieved in the current study via greater calorie restriction. Although 60% to 70% restriction has been achieved in controlled weight loss studies conducted in the absence of pasture consumption, this was unobtainable because of practical limitations. For example, some owners were unable to completely remove pasture from the horses' diet for managerial reasons leading to an unquantifiable amount of calories coming from pasture. Furthermore, each horse was receiving a different type of hay, grain, or supplement based on the 17 different field conditions (privately owned stables) in this study. The dietary protocols that the researchers created for the owners took into account the horse's currently available diet instead of switching all horses to the same diet. This represents a practical scenario in which owners are often limited by hay availability or by boarding stable feeding and management accommodations. In addition, consideration to animal safety was of primary importance because the diets were carried out by owners and barn managers.

Despite considerable efforts for similarity in dietary restriction protocols, there was substantial variation in weight loss between horses. Nutritional and management information collected at the final consultations yielded possible explanations for the heterogeneity in weight loss observed, primarily pertaining to owner's adherence to the weight loss protocol and possible metabolic differences between horses. It is possible that the 15% restriction mean generated from the revised diets was generally lower based on the level of compliance with the weight loss protocols. It was observed that some owners exhibited difficulty in adhering to the protocol over a change of seasons and pasture availability. It was noted that some owners increased supplemental hay

provisions in response to dwindling pasture reserves over winter months (Moffitt et al. 1987). This increase in provisions was occasionally unreported by owners until the final consultation. Although owners were asked to restrict their horses' pasture intake by either providing turnout in a dry lot or by using a grazing muzzle, this was not always feasible based on facility accommodations. It appeared that the amount of time on pasture significantly affected the amount of weight loss achieved in the horses. In general, horses receiving unrestricted pasture were grouped in the continuous pasture intake group, given lower owner compliance scores, and lost the least amount of weight. Continuous pasture access may lead to overeating in horses because it is estimated that horses may consume pasture at a rate of 2.4% BW/d (Chavez et al. 2014, Chenost and Martin-Rosset 1985, Dulphy et al. 1997, Fleurance et al. 2001, Grace et al. 2002, Marlow et al. 1983, Siciliano 2012). Horses with no access to pasture lost the greatest amount of BW and BCS. Based on the results of this study, partial pasture access provided better restriction of caloric intake than continuous pasture access; however, horses with time-restricted access to pasture may increase their intake rate when time at pasture is restricted, if not wearing a grazing muzzle (Dowler 2009, Ince et al. 2011). Overall, no access to pasture, which was recommended for many horse owners, proved to be the most effective method for BW loss, which enabled owners to have the most control over their horse's intake level.

There was a wide range of owner compliance observed in this study. Although owners elected to participate in the study, some owners participated very little in the study, while others exhibited a vested interest in following the researcher's instructions. Therefore, it is no doubt that compounding factors, such as owner

compliance with the weight loss protocols, may have contributed to the variation in overall  $\Delta$ BW seen in the client-owned horses. It is likely that owners scoring a compliance level of 1 or 2 did not feed the 80% to 90% DEI restriction that was recommended to them at the beginning of the trial period. Owners who minimally restricted intake in their horses incurred less of a change in BW and BCS in their horses than owners that complied well with the weight loss protocols. The fact that there was still weight loss in horses with non-compliant owners meant that there may have been seasonal factors that facilitated weight loss in these pasture-based horses (i.e., pasture grass availability and reduction in supplemental feeding of hay, metabolic thermoregulation, voluntary or involuntary activity, and so forth), especially since the control horses on continuous pasture and supplemental hay gained body weight and condition overall.

Overall, it was observed that good compliance (level 3) was associated with owners who had knowledge about obesity/obesity-related illness and/or were receiving veterinary treatment for such issues, and these horses lost the greatest amount of weight overall. Studies by Yaissle et al. (2004) and Bissot et al. (2010) found that owners were more willing to follow the dietary plans once they noticed an increase in their animal's activity level and a decrease in body condition score. It is possible that compliant owners may have achieved greater weight loss in their horses because of motivation to continue the diet after seeing positive results. Other weight loss studies in dogs reported providing information and education materials, food and veterinary care at no or low cost, and weight checks to increase compliance (Gentry 2006, Laflamme and Kuhlman 1995, Lund et al. 2006). Perhaps providing owners with educational

materials would have increased compliance efforts in the current study. Although owners were given weight tapes, no monthly record of weight measurements were required, but may have been an effective motivation tool to maintain continued compliance.

Good owner compliance with the weight loss protocol was related to the exclusion of pasture access in their horses. However, some continuous grazing horses had moderately compliant owners who restricted grazing by turning them out on a sparse pasture and/or by restricting supplemental rations to achieve close to the desired level of digestible energy intake. Nevertheless, there was considerable variation in weight loss for horses in the no pasture group and/or with fully compliant owners. This may have been associated with differences in breed, age, basal metabolism, and/or voluntary activity level. Differences in metabolism may have contributed to the between-horse variability seen in previous weight loss studies (Argo et al. 2012, Dugdale et al. 2010, Ungru et al. 2012). Individual variation in the rate of weight loss in response to dietary restriction is recognized in human and murine subjects (Bouchard and Tremblay 1997, Rikke et al. 2006, Wang and Lui 2008). It appears that genetic variation may play a significant role in obesity predisposition (Geor 2008, Treiber et al. 2006). Argo and colleagues (2012) reported different rates of weight loss in 'diet-sensitive' verses 'diet-resistant' animals, which was mainly found to attribute to the animal itself and was not associated with any onset variables, such as diet, sex, breed, BCS, body mass index, indices of insulin resistance, body composition, or subcutaneous fat depth. Perhaps, variation in metabolic differences between horses plays an

important role in the effectiveness of dietary restriction and warrants further investigation.

There were managerial factors that affected gaining full compliance from owners for this study. Owners reported difficulty with following the protocols due to individual horse preference and adverse behaviors. Some owners reported complications in restricting feed (either by inadequate facility management or uncooperative attitude of the horse) or the use of grazing muzzles (unable to keep on horse or unable to catch horse to apply muzzle). Although owners were asked to weigh rations, this may have not been accomplished continuously during the duration of the study as owners got accustomed to the feeding regimen. It is unlikely that the horse owners/managers would conduct the study with as much diligence as would a researcher, which is why this study reflects the practical nature of weight management in the field.

Approximately half of owners given grazing muzzles did not use them or were inconsistent in their use. This alone provides evidence that owner education on the effectiveness of grazing muzzles needs to be emphasized. However, some facilities were able to accommodate husbandry modifications, such as creating dry lots, weighing feeds, and using a grazing muzzle when at pasture, which was recorded in email conversations with the researchers. These horses exhibited the highest amount of weight loss and greatest decrease in body condition score.

Change in BCS was consistent with the result from similar duration studies ( $1.5 \pm 0.3$ ; 20 weeks); however, shorter studies reported less of a  $\Delta$ BCS ( $0.4 \pm 0.8$ ; 6 weeks and  $0.3 \pm 0.1$ ; 12 weeks) (Argo et al. 2012, Dugdale et al. 2010, McGowan et al. 2013). Consequently, the discrepancy between BCS and BW may be due to only visceral

adipose tissue being depleted more readily than subcutaneous adipose tissue, affecting observable results in BCS. Therefore, the trial length of this study may have provided enough time for  $\Delta$ BCS to reasonably correlate with  $\Delta$ BW (%). Morphometric measures of HG and NC decreased overall, in agreement with previous literature (Argo et al. 2012, Dugdale et al. 2010).

High basal GLU concentrations have been observed in obese horses before weight loss (Frank et al. 2006). With decreasing weight, basal GLU was not affected and remained fairly constant overall, which was in agreement with the literature (Argo et al. 2012, Bailey and Bamford 2013, McGowan et al. 2013, Place et al. 2010). Even with minimal declines in BW, basal INS significantly decreased, however was not influenced by basal GLU. Freestone and colleagues (1992) found that obese ponies, losing only 3.1% BW displayed significant reductions in basal INS, whereas basal GLU remained unaffected. Other weight loss studies have reported minimal reductions in weight may reflect significant reductions in basal INS (Dugdale et al. 2010, Van Weyenberg et al. 2008). Because prolonged hyperinsulinemia has clear implications towards laminitis in horses and ponies, the reduction in basal INS via dietary-mediated weight reduction is likely advantageous to horses predisposed to laminitis. King and Mansmann (2004) reported that weight reduction using dietary modification usually takes precedence in the recovery process in overweight laminitic horses that are in the early stages of the recovery process.

There were a few limitations to this study that should be noted. Since the use of a portable electronic scale was unobtainable, the authors had to resort to the next best method of weight estimation that was available at the time, which was the weight

calculation equation described by Carroll and Huntingdon (1988). Therefore, it is safe to assume that the dietary intake and dietary adjustments was approximate but not exact. For future study, the weight estimation that accounts for both neck circumference and belly circumference may provide a more precise measurement of body weight, when an electronic scale is unavailable. In addition, because of the nature of the study, whereby owners were given dietary protocols for weight loss but were not regularly monitored (because of limited personnel and time), the exact calories consumed by each horse per day are unavailable. Similarly, pasture intake was not directly quantified, and therefore, values of DE intake from pasture were estimates. Nonetheless, this study generated data from a practical setting where owners were given nutritional recommendations and were expected to follow the directions accordingly.

Feeding 80% to 90% of digestible energy requirement in overweight horses that were nutritionally managed by their owners resulted in approximately 6% reduction in BW and a significant decrease in BCS, morphometric measurements, and basal INS. Although a multitude of managerial situations occurred, this study provided evidence that restricted grazing through the use of a dry lot or a grazing muzzle and the owner's ability to fully comply with the feeding protocol were essential for weight reduction. Furthermore, the data suggests that modest levels of weight reduction may improve basal INS in horses. Although owner education seems essential for weight management in horses, even compliant owners may struggle with reducing the weight of their horses if the horse has a low basal metabolic rate. Therefore, investigation into the metabolic

differences in horses predisposing them to obesity and a resistance to weight loss is of potential interest in the future.

Table 4. Phenotypic Summary of the Horses in this Study

| Horses        | Breed           | Gender  | Age (yr)       | BW (kg)            | Height (cm)   | BCS           |
|---------------|-----------------|---------|----------------|--------------------|---------------|---------------|
| 1             | Paso Fino       | Mare    | 12             | 423                | 142           | 8.25          |
| 2             | Paint Horse     | Gelding | 10             | 501                | 153           | 8.00          |
| 3             | QH x Morgan     | Gelding | 15             | 405                | 144           | 7.75          |
| 4             | Welsh           | Gelding | 8              | 391                | 142           | 7.50          |
| 5             | Holsteiner      | Gelding | 14             | 617                | 173           | 7.00          |
| 6             | Quarter Horse   | Gelding | 13             | 522                | 145           | 7.00          |
| 7             | Halflinger x QH | Mare    | 8              | 484                | 144           | 8.75          |
| 8             | Appaloosa       | Gelding | 16             | 735                | 154           | 8.75          |
| 9             | Paint Horse     | Gelding | 12             | 525                | 144           | 8.00          |
| 10            | Paint Horse     | Mare    | 15             | 570                | 152           | 7.50          |
| 11            | Quarter Horse   | Mare    | 11             | 617                | 152           | 7.25          |
| 12            | Quarter Horse   | Mare    | 17             | 552                | 145           | 8.00          |
| 13            | Appaloosa       | Gelding | 12             | 569                | 153           | 7.25          |
| 14            | Halflinger      | Mare    | 14             | 552                | 144           | 8.00          |
| 15            | Hanoverian      | Gelding | 11             | 585                | 163           | 7.50          |
| 16            | Icelandic Horse | Gelding | 11             | 411                | 134           | 8.75          |
| 17            | Quarter Horse   | Gelding | 3              | 558                | 154           | 7.75          |
| 18            | Quarter Horse   | Mare    | 10             | 553                | 153           | 7.50          |
| 19            | QH x Arab       | Mare    | 7              | 409                | 144           | 7.50          |
| 20            | Paint Horse     | Mare    | 17             | 558                | 153           | 7.50          |
| 21            | Mustang         | Mare    | 9              | 487                | 143           | 8.00          |
| 22            | Hanoverian      | Mare    | 10             | 625                | 163           | 7.75          |
| 23            | Paint Horse     | Mare    | 14             | 570                | 154           | 7.00          |
| 24            | Appaloosa x TB  | Gelding | 10             | 515                | 154           | 6.50          |
| Mean $\pm$ SE |                 | 12G:12M | 11.6 $\pm$ 0.7 | 530.71 $\pm$ 14.87 | 150 $\pm$ 1.7 | 7.7 $\pm$ 0.1 |

Table 5. Summary of Data Describing the Mean Physical Measurements of Client-Owned Horses at the Beginning and End of the Trial Period (184 ± 28 days)

| Client owned | Initial        | Final          | p value |
|--------------|----------------|----------------|---------|
| BW (kg)      | 530.71 ± 14.87 | 505.30 ± 14.84 | < 0.01  |
| BCS          | 7.7 ± 0.1      | 6.3 ± 0.2      | < 0.01  |
| CNS          | 2.5 ± 0.2      | 1.5 ± 0.3      | < 0.01  |
| NC (cm)      | 104.60 ± 1.80  | 100.00 ± 1.78  | < 0.01  |
| HG (cm)      | 193.82 ± 1.88  | 189.78 ± 1.86  | < 0.01  |

Abbreviations: BW, body weight; BCS, body condition score; CNS, cresty neck score; NC, neck circumference; HG, heart girth.

Values represent mean ± standard error of the mean. Significance was accepted at  $p < 0.05$ .

Table 6. Summary of Data Describing the Control Horse Mean Physical Measurements at the Beginning and End of the Trial Period (n=4)

| Control | Initial   | Final     | p value |
|---------|-----------|-----------|---------|
| BW (kg) | 500 ± 57  | 562 ± 47  | 0.027   |
| BCS     | 7.4 ± 0.2 | 8.1 ± 0.2 | 0.089   |
| CNS     | 1.1 ± 0.1 | 1.4 ± 0.2 | 0.18    |
| NC (cm) | 98 ± 5    | 109 ± 4   | 0.022   |
| HG (cm) | 190 ± 8   | 202 ± 5   | 0.032   |

Abbreviations: BW, body weight (scale); BCS, body condition score; CNS, cresty neck score; NC, neck circumference; HG, heart girth.

Values represent the mean ± standard error of mean. Significance was accepted at  $p < 0.05$ .

Table 7. Revised Dietary Recommendations for the Horse Owners

| Horse | Revised diet (daily)  |   |  | Revised DE intake <sup>a</sup> |
|-------|---|---|--|--------------------------------|
|       | Concentrate (per day)   | Hay (per day)                               | Pasture (per day)  | Kcal/kg/d                      |
| 1     | 0.30-kg Omelene 300, supplements  | 5.0-kg (2 flakes) Timothy hay               | 1-hr turnout with a grazing muzzle                                 | 31.3                           |
| 2     | 50-g vitamin/mineral supplement   | 3.62 (2 flakes) Timothy mix hay             | 24-hr turnout with a grazing muzzle                                | 36.8                           |
| 3     | 50-g vitamin/mineral supplement   | 7.24 (4 flakes) Timothy mix hay             | 24-hr turnout on dry lot or with a grazing muzzle                  | 24.4                           |
| 4     | 50-g vitamin/mineral supplement   | 8.0-kg (4 flakes) Coastal hay               | 12-hr turnout with a grazing muzzle                                | 40.6                           |
| 5     | 1.0- kg 14/6 grain; 50-g vitamin/mineral supplement; 1.0-kg alfalfa pellets | 6.0-kg (3 flakes) Coastal hay               | 12-hr turnout with a grazing muzzle                                | 36.6                           |
| 6     | 50-g vitamin/mineral supplement   | 4.0-kg hay (2 flakes) Coastal hay           | 12-hr turnout with grazing muzzle                                  | 23.5                           |
| 7     | 50-g vitamin/mineral supplement; 100 g of beet pulp                         | 2.0-kg (4 flakes) Coastal hay               | 12-hr turnout with grazing muzzle                                  | 23.8                           |
| 8     | 50-g vitamin/mineral supplement   | None  | 24-hr turnout on limited pasture; grazing muzzle applied for 12 hr | 23.9                           |
| 9     | 50-g vitamin/mineral supplement; 170-g Tribute LS                           | 4.0-kg (2 flakes) grass hay                 | 24-hr turnout with grazing muzzle                                  | 25.9                           |
| 10    | 50-g vitamin/mineral supplement; 170-g Tribute LS                           | 4.0- kg (2 flakes) grass hay                | 24-hr turnout with grazing muzzle                                  | 26.2                           |
| 11    | 50-g vitamin/mineral supplement   | 4.89-kg (3 flakes) Fescue/Orchard grass hay | 12-hr limited pasture access                                       | 24.9                           |
| 12    | 50-g vitamin/mineral supplement   | 4.89-kg (3 flakes) Fescue/Orchard grass hay | 12-hr turnout with grazing muzzle                                  | 25.6                           |
| 13    | 50-g vitamin/mineral supplement   | 4.89-kg (3 flakes) Fescue/Orchard grass hay | 12-hr limited pasture access                                       | 27.7                           |
| 14    | 270-g Sunshine Plus ration balancer   | 4.5-kg (2 flakes) Coastal hay               | 24-hr in a dry lot   | 23.6                           |
| 15    | 250-g Triple Crown ration balancer  | 6.0-kg (3 flakes) Brome hay                 | 12 hr turnout with grazing muzzle                                  | 28.3                           |

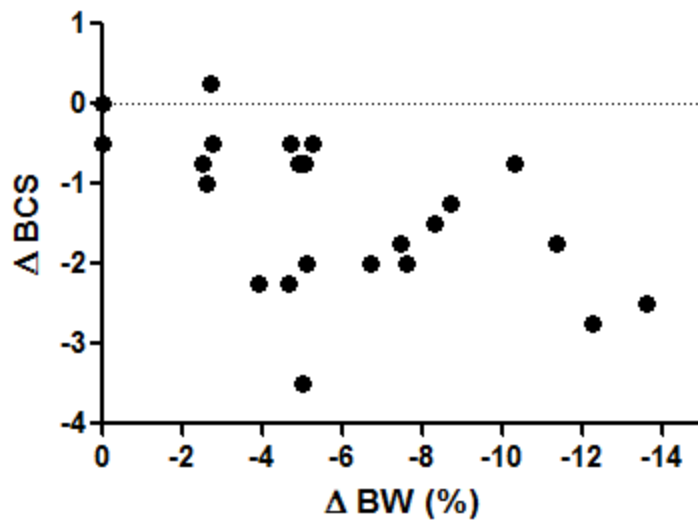
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3 Table 7. Continued

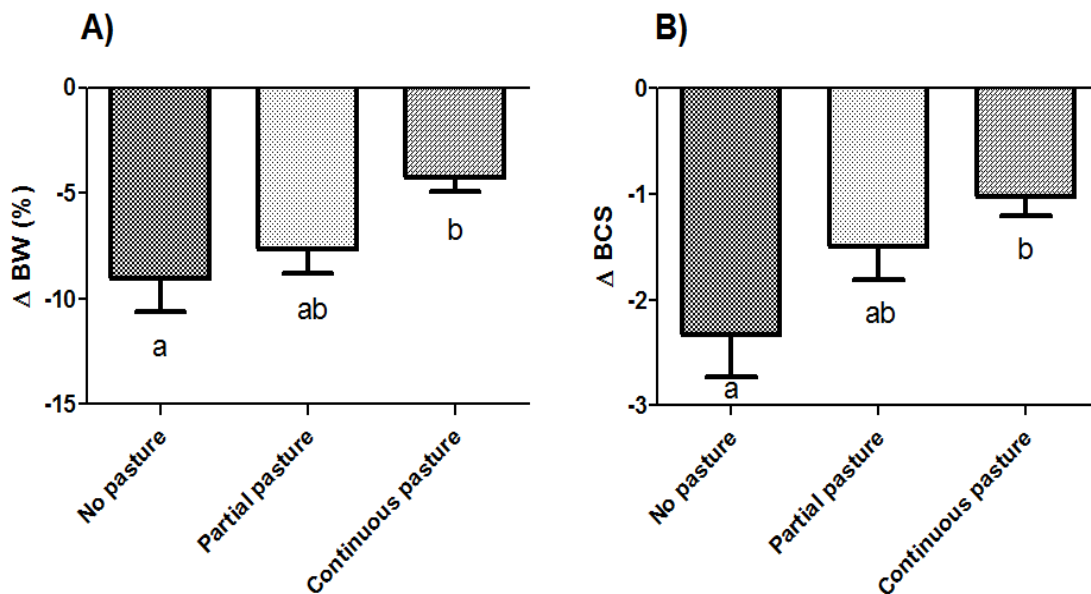
|    |   |  |  |      |
|----|---|--|--|------|
| 16 | 50-g vitamin/mineral supplement   | None   | 12 hr turnout with grazing muzzle          | 24.7 |
| 17 | 50-g vitamin/mineral supplement   | 3.3-kg (2 flakes) Timothy hay                                    | 12-24 hr turnout on sparse/dry lot pasture | 29.9 |
| 18 | 50-g vitamin/mineral supplement   | None   | 24 hr turnout on limited pasture           | 29.8 |
| 19 | 50-g vitamin/mineral supplement   | None   | 12 hr turnout with grazing muzzle          | 32.7 |
| 20 | 50-g vitamin/mineral supplement   | 3.0-kg (2 flakes) Orchard grass hay                              | 12 hr turnout with grazing muzzle          | 25.5 |
| 21 | 50-g vitamin/mineral supplement; 100-g beet pulp; 100-g alfalfa pellets | 2.0-kg (2 flakes) Coastal hay                                    | 24 hr turnout with grazing muzzle          | 26.1 |
| 22 | 50-g vitamin/mineral supplement; 300-g Sentinel LS; supplements         | 8.0-kg (5 flakes) Orchard grass hay                              | dry lot                                    | 30.8 |
| 23 | 900-g Purina Healthy Edge   | 7.56-kg (4 flakes) Timothy/Orchard hay                           | 24 hr turnout on sparse/dry lot pasture    | 33.5 |
| 24 | 460-g 12/6 grain; 40-g vitamin/mineral supplement                       | 6.0-kg (3 flakes) Teff hay; 2.48-kg (2 flakes) of clover mix hay | 24 hr turnout on sparse/dry lot pasture    | 28.0 |

Abbreviation: DE, digestible energy.

<sup>a</sup> Some of the estimates include the National Research Council (2007) requirement for horses in light work.

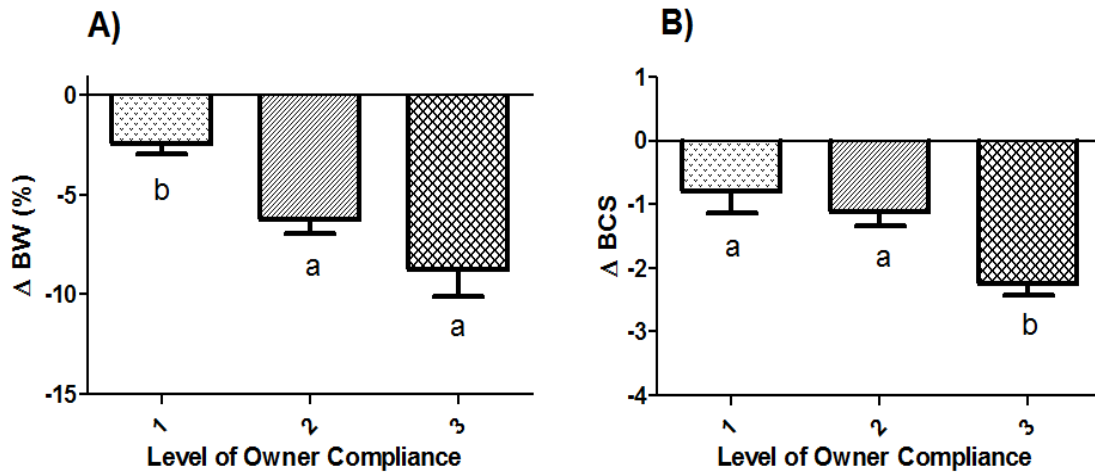


**Figure 2.** Change in body condition score (BCS) was regressed on concurrently recorded change in body weight (BW) as a percent of initial.



**Figure 3.** A) Mean change in body weight (BW; %) and B) body condition score (BCS) in horses when grouped according to no pasture (n=6), partial pasture (n=4), and continuous pasture (n=13).

<sup>a,b</sup> Different superscripts indicate treatment differences ( $p < 0.05$ ).



**Figure 4.** A) Mean change in body weight (BW; %) and B) body condition score (BCS) in horses when grouped according to owner compliance level 1 (n=6), 2 (n=10), and 3 (n=7).

<sup>a,b</sup> Different superscripts indicate treatment differences (p<0.05).

#### Literature Cited

- Thatcher, C. D., R. S. Pleasant, R. J. Geor, F. Elvinger. 2012. Prevalence of overconditioning in mature horses in southwest Virginia during the summer. *J. Vet. Intern. Med.* 26(6): 1413-1418.
- Dugdale, A. H. A., G. C. Curtis, P. Cripps, P. A. Harris, C. M. Argo. 2010. Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Vet. J.* 42(7): 600-610.
- Pratt-Phillips, S.E., K. M. Owens, L. E. Dowler, M. T. Cloninger. 2010. Assessment of resting insulin and leptin concentrations and their association with managerial and innate factors in horses. *J. Equine Vet. Sci.* 30(3):127-133.
- Wyse, C. A., K. A. McNie, V. J. Tannahill, J. K. Murray, S. Love. 2008. Prevalence of obesity in riding horses in Scotland. *Vet. Rec.* 162(18):590-591.
- Harker, I. J., P. A. Harris, C. F. Barfoot. 2011. The body condition score of leisure horses competing at an unaffiliated championship in the UK. *J. Equine. Vet. Sci.* 31(5):253-254.
- Gossellin, J., J. A. Wren, S. J. Sunderland. 2007. Canine obesity-an overview. *J. Vet. Pharmacol. Ther.* 30(S1):1-10.
- Suagee, J.K., B. A. Corl, M. V. Crisman, B. S. Pleasant, C. D. Thatcher, R. J. Geor. 2013. Relationships between body condition score and plasma inflammatory cytokines,

insulin, and lipids in a mixed populations of light-breed horses. *J. Vet. Intern. Med.* 27(1): 157–163.

Ungru, J., M. Blüher, M. Coenen, J. Raila, R. Boston, I. Vervuert. 2012. Effects of body weight reduction on blood adipokines and subcutaneous adipose tissue adipokine mRNA expression profiles in obese ponies. *Vet. Rec.* 171(21): 528.

Geor, R.J. 2010. Nutrition and exercise in the management of horses and ponies at high risk for laminitis. *J. Equine Vet. Sci.* 30(9): 463–470.

Hoffman, R.M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *J. Anim. Sci.* 81(9): 2333–2342.

Geor, R. J. 2008. Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *J. Equine Vet. Sci.* 28(12): 753–759.

Frank, N., R. J. Geor, S. R. Bailey, A. E. Durham, P. Johnson. 2010. Equine metabolic syndrome. *J. Vet. Intern. Med.* 24(3): 467–475.

Piya, M. K., P. G. McTernan, S. Kumar. 2013. Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *J. Endocrinol.* 216(1): T1–T15.

Bailey, S.R., N. J. Bamford. 2013. Metabolic responses of horses and ponies to high and low glycaemic feeds: implications for laminitis. *Anim. Prod. Sci.* 53(11): 1182–1187.

Holbrook, T. C., T. Tipton, D. McFarlane. 2012. Neutrophil and cytokine dysregulation in hyperinsulinemic obese horses. *Vet. Immunol. Immunopathol.* 145(1): 283–289.

Vick, M. M., A. A. Adams, B. A. Murphy, D. R. Sessions, D. W. Horohov, R. F. Cook, B. J. Shelton, B. P. Fitzgerald. 2007. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. *J. Anim. Sci.* 85(5): 1144–1155.

McCue, M. E., R. J. Geor, N. Schultz. 2015. Equine metabolic syndrome: a complex disease influenced by genetics and the environment. *J. Equine Vet. Sci.* 35(5): 367–375.

Johnson, J. P., C. E. Wiedmeyer, N. T. Messer, V. K. Ganjam. 2009. Medical implications of obesity in horses-lessons for human obesity. *J. Diabetes Sci. Technol.* 3(1): 163–174.

Treiber, K. H., D. S. Kronfeld, R. J. Geor. 2006. Insulin resistance in equids: possible role in laminitis. *J. Nutr.* 136(7): 2094S–2098S.

King, C., R. A. Mansmann. 2004. Preventing laminitis in horses: dietary strategies for horse owners. *Clin. Tech. Equine Pract.* 3(1):96–102.

Geor, R. J. 2010. Current concepts on the pathophysiology of pasture-associated laminitis. *Vet. Clin. North Am. Equine Pract.* 26(2): 265–276.

Argo, C. M., C. G. Curtis, D. Grove-White, A. H. A. Dugdale, C. F. Barfoot, P. A. Harris. 2012. Weight loss resistance: a further consideration for the nutritional management of obese Equidae. *Vet. J.* 194(2): 179–188.

McGowan, C. M., A. H. A. Dugdale, G. L. Pinchbeck, C. M. Argo. 2013. Dietary restriction in combination with a nutraceutical supplement for the management of equine metabolic syndrome in horses. *Vet. J.* 196(2): 153–159.

Van Weyenberg, S., M. Hesta, J. Buyse, G. P. J. Janssens. 2008. The effect of weight loss by energy restriction on metabolic profile and glucose tolerance in ponies. *J. Anim. Physiol. Anim. Nutr.* 92(5): 538–545.

Lawrence, L. M., Cymbaluk, N. F., D. W. Freeman, R. J. Geor, P. M. Graham-Thiers, A. C. Longland, et al. 2007. Nutrient requirements of horses. 6th ed. The National Academies Press, Washington DC.

Dugdale, A. H. A., G. J. Curtis, P. J. Cripps, P. A. Harris, C. M. Argo. 2011. Effects of season and body condition on appetite, body mass and body composition in ad libitum fed pony mares. *Vet. J.* 190(3): 329–337.

Lund, E. M., P. J. Armstrong, C. A. Kirk, J. S. Klausner. 2006. Prevalence and risk factors for obesity in adult dogs from private US veterinary Practices. *Intern. J. Appl. Res. Vet. Med.* 4(2): 177.

Bouchard, C., A. Tremblay. 1997. Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. *J. Nutr.* 127(5): 943S–947S.

Wang, Y., J. Liu. 2008. Plasma ghrelin modulation in gastric band operation and sleeve gastrectomy. *Obes. Surg.* 19(3): 357–362.

Gentry, S. J. 2006. Results of the clinical use of a standardized weight reduction program in dogs and cats. *J Am Anim Hosp Assoc.* 29():369–375.

Laflamme, D. P., G. Kuhlman. 1995. The effect of weight loss regimen on subsequent weight maintenance in dogs. *Nutr. Res.* 15(7): 1019–1028.

German, A. J., S. L. Holden, T. Bissot, R. M. Hackett, V. Biourge. 2007. Dietary energy restriction and successful weight loss in obese client-owned dogs. *J. Vet. Intern. Med.* 21(6): 1174–1180.

Carter, R. A., R. J. Geor, W. B. Stanier, T. A. Cubitt, P. A. Harris. 2009. Apparent adiposity assessed by standardized scoring systems and morphometric measurements in horses and ponies. *Vet. J.* 179(2):204–210.

Henneke, D., G. Potter, J. Kreider, B. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15(4): 371–372.

Carroll, C. L., P. J. Huntingdon. 1988. Body condition scoring and weight estimation in horses. *J. Equine Vet. Sci.* 20(1):41–45.

Pagan, J. D. 1998. Nutrient digestibility in horses. *Adv. Equ. Nutr.* 77–83.

Longland, A. C., C. Barfoot, P. A. Harris. 2012. The effect of wearing a grazing muzzle vs. not wearing a grazing muzzle on intakes of spring, summer and autumn pastures by ponies. in: M. Saastamoinen, M.J. Fradinho, A.S. Santos, N. Miraglia (Eds.) *Forages and grazing in horse nutrition*. Wageningen Academic Publishers, The Netherlands, 185–186.

Dowler, L. E., P. D. Siciliano, S. E. Pratt-Phillips, M. Poore. 2012. Determination of pasture dry matter intake rates in different seasons and their application in grazing management. *J. Equine Vet. Sci.* 32(2): 85–92.

Reimers, T. J., R. G. Cowan, J. P. McCann, M. W. Ross. 1982. Validation of a rapid solid-phase radioimmunoassay for canine, bovine, and equine insulin. *Am. J. Vet. Res.* 43(7): 1274–1278.

Motulsky, H. J. 2007. Prism 5 statistics guide. GraphPad Software Inc, San Diego, CA.

Walker, G. A. 2002. Common statistical methods for clinical research. 2nd. SAS Institute, Inc, Cary, NC.

Bissot, T., E. Servet, S. Vidal, M. Deboise, R. Sergheraert, G. Egron, M. Hugonnard, S. E. Heath, V. Biourge, A. J. German. 2010. Novel dietary strategies can improve the outcome of weight loss programmes in obese client-owned cats. *J. Feline Med. Surg.* 12(2): 104–112.

Moffitt, D. F., T. N. Meacham J. P. Fontenot V. G. Allen. 1987. Seasonal differences in apparent digestibility of fescue and orchard grass/clover pastures in horses. In: *proceedings of the 10th equine nutrition and physiology symposium, 11-13 June, Fort Collins Marriot, Fort Collins, CO*, 79.

Dulphy, J. P., W. Martin-Rosset, H. Dubroeuq, J. M. Ballet, A. Detour, M. Jailer. 1997. Compared feeding patterns in ad libitum intake dry forages in horses and sheep. *Livest. Prod. Sci.* 52(1): 49–56.

Grace, N., E. Gee, E. Firth, H. Shaw. 2002. Digestible energy intake, dry matter digestibility and mineral status of grazing New Zealand Thoroughbred yearlings. *NZ Vet. J.* 50(2): 63–69.

Fleurance, G., P. Duncan, B. Mallevaud. 2001. Daily intake and the selection of feeding sites by horses in heterogeneous wet grasslands. *Anim. Res.* 50(2): 8.

Marlow, C. H. B., E. M. van Tonder, F. C. Hayward, S. S. van der Merwe, L. E. G. Price. 1983. A report on the consumption, composition, and nutritional adequacy of a mixture of lush green perennial ryegrass (*Lolium perenne*) and crocksfoot (*Dactylis glomerata*) fed ad libitum to Thoroughbred mares. *J. S. Afr. Vet. Assoc.* 54(3): 155–157.

Chavez, S. J., P. D. Siciliano, G. B. Huntington. 2014. Intake estimation of horses grazing tall fescue (*Lolium arundinaceum*) or fed tall fescue hay. *J. Anim. Sci.* 92(5): 2304–2308.

Chenost, M., W. Martin-Rosset. 1985. Comparison between species (sheep, horse, cattle) on intake and digestibility of fresh herbage. *Ann. Zootech.* 34: 291–312.

Siciliano P. D. 2012. Estimation of pasture dry matter intake and its practical application in grazing management for horses. *Proc. 10th Mid-Atlantic Nutr. Conf.*, Timonium, MD.

Ince, J., A. C. Longland, J. C. Newbold. 2011. Changes in proportions of dry matter intakes in ponies with access to pasture and haylage for 3-20 hours per day, respectively, for 6 weeks. *J. Equine Vet. Sci.* 31(5): 283.

Dowler L. E. 2009. Prediction of hourly dry matter intake in horses; [MS Thesis] Animal Science Department at North Carolina State University, Raleigh, NC.

Yaissle, J. E., C. Holloway, C. A. T. Buffington. 2004. Evaluation of owner education as a component of obesity treatment program for dogs. *J. Am. Vet. Med. Assoc.* 224(12): 1932–1935.

Rikke, B. A., M. E. Battaglia, D. B. Allison, T. E. Johnson. 2006. Murine weight loss exhibits significant genetic variation during dietary restriction. *Physiol. Genomics.* 27(2): 122–130.

Frank, N., B. E. Sarah, E. B. Laura, H. K. Duane. 2006. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J. Am. Vet. Med. Assoc.* 228(9): 1383–1390.

Place, N. J., C. M. McGowan, S. V. Lamb, B. J. Schanbacher, T. McGowan, D. M. Walsh. 2010. Seasonal variation in serum concentrations of selected metabolic hormones in horses. *J. Vet. Intern. Med.* 24(3): 650–654.

Freestone, J. F., R. Beadle, K. Shoemaker, R. T. Bessin, K. J. Wolfsheimer, C. Church. 1992. Improved insulin sensitivity in hyperinsulinaemic ponies through physical conditioning and controlled feed intake. *Equine Vet. J.* 24(3): 187–190.

Asplin, K. E., M. N. Silience, C. C. Pollitt, C. M. McGowan. 2007. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *Vet. J.* 174(3):530–535.

De Laat, M. A., C. M. McGowan, M. N. Silience, C. C. Pollitt. 2010. Equine laminitis: induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine Vet. J.* 42(2): 129–135.

## CHAPTER III: EXPERIMENT 2

### **Relationships among digestible energy intake, body weight, and body condition in mature idle horses**

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#### 3.1 Abstract

The objective of this study was to evaluate the relationships among digestible energy (DE) intake, body weight and body condition in lean/moderate condition horses fed above maintenance calorie intakes. The feed intake of 35 Quarter Horses (age  $5.3 \pm 1.2$  yr; BW  $462 \pm 39$  kg; BCS  $4.0 \pm 1.0$ ) was recorded daily in three 42-d feeding trials. Horses were offered 1.75 to 2.00 kg DM/100 kg BW in mixed grass hay and 0.2 kg/100 kg BW in whole oats. Scale body weight and body condition score (BCS) was recorded every 2 wk. Daily DE intake was calculated as the amount fed minus any refusal, multiplied by the DE (Mcal/kg) content of the feeds. The mean within horse  $\Delta$ BW and  $\Delta$ BCS from 0 to 42 d were evaluated using paired t-tests. Trial 1 horses consumed  $53.21 \pm 0.51$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> and BW increased ( $p < 0.001$ ) by  $24 \pm 3$  kg; however, there was no significant ( $p=0.43$ ) change in BCS ( $0.0 \pm 0.5$ ) from d 0 to d 42. Trial 2 horses consumed  $47.68 \pm 0.51$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> and BW increased ( $p=0.009$ ) by  $8 \pm 3$  kg and BCS tended to increase ( $p=0.06$ ) by  $0.5 \pm 0.5$  from d 0 to d 42. Trial 3 horses consumed  $51.22 \pm 0.53$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> and BW and BCS increased ( $p<0.001$ ) by  $22 \pm 2$  kg and  $1.0 \pm 0.5$ , respectively, from d 0 to d 42. For all three trials the mean DE intake was  $51.73 \pm 3.33$  Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> ( $23.83 \pm 0.36$  Mcal/d) and the horses required  $24 \pm 3$  Mcal/kg of BW gain.

### 3.2 Introduction

There are important welfare concerns for horses that are significantly underweight (BCS <2.5/9) or overweight (BCS >7/9) (Geor 2008, Kronfeld 1993, Muñoz et al. 2010, Stull 2009, Suagee et al. 2013). Several studies have investigated feeding regimens for such horses to recover from starvation (Witham and Stull 1998), and for overweight horses to lose weight (Argo et al. 2012, Dugdale et al. 2010, Geor and Harris 2009, Gill et al. 2016, McGowan et al. 2013). The relationships among DE intake, body weight, and body condition has been described in horses being fed towards obesity (Carter et al. 2009, Lindåse et al. 2016); however, there is limited data describing the relationship between DE intake and body weight change in lean/moderate (BCS 3-5/9) horses. Such data is important to add to the pool of knowledge about calorie intakes required for gain; a measure that has the potential to show considerable variation based on animal-related factors (i.e., maturity, initial body condition, fitness) and feed composition (Bush et al. 2001, Cyambaluk et al. 1989, National Research Council 2007, Potter et al. 1990). The present study reports the energy intake of 35 horses over a 42-d period to provide insight into the relationships among digestible energy intake, body weight, and body condition score in idle horses.

### 3.3 Materials and Methods

The following experimental protocol was approved by the North Carolina State University Institutional Animal Care and Use committee (No. 13-126-O).

#### *3.3 a. Animals and Husbandry*

The experiment was conducted in three separate trials from March 25th, 2014 to August 18th, 2014 at the North Carolina State University Equine Research Unit in

Raleigh, North Carolina. Thirty-five mature, idle, registered Quarter Horse geldings (mean  $\pm$  standard deviation;  $5.3 \pm 1.2$  years;  $462 \pm 39$  kg; BCS  $4.5 \pm 1$ ) participated in a 42-d research study. Horses had unknown backgrounds except the researchers were provided with pedigrees.

Because of space limitations in the research facility, horses were split into three consecutive feeding trials. There were twelve horses in trial 1, twelve horses in trial 2, and eleven horses in trial 3. The horses in each trial were housed together in a large, dry lot pen with a lean-to shelter and free access to hay and water for approximately 14 d upon arrival to the facility, before the start of the study.

Following the 14-d acclimation period, the horses were housed in individual, partially covered,  $3.7 \text{ m} \times 12.2 \text{ m}$ , dry lot pens for 7 d prior to d-0 and for the remainder of the 42-d trial period. Each dry lot pen was equipped with an automatic watering trough, a 5-gallon feed bucket, and a hay bin. The pens were cleaned twice daily.

Horses were maintained on a preventative health protocol, which included hoof care, dental exams, teeth floating, and deworming, that were performed within 7 d prior to d-0 of each trial period. Horses were groomed weekly and hooves were picked out every other day.

Scale BW and BCS was conducted at 7:00 am on d 0, 13, 27, and 41 using an electronic large animal scale with  $\pm 1.0$  kg sensitivity (Smart Scale 200, Gallagher Animal Management, USA). Body condition scores were determined according to the 1 to 9 point scale (Henneke et al. 1983) and performed by 2 experienced equine professionals (using whole units). The scores were averaged and represented in the nearest half or whole unit.

### *3.3 b. Dietary Protocol*

Horses were fed weighed amounts of mixed grass hay and whole oats twice daily. The nutrient composition of the mixed grass hay and whole oats for each trial are shown in Tables 8-10. Trial 1 horses were offered 2.00 kg DM/100 kg BW in mixed grass hay and 0.2 kg DM/100 kg BW in whole oats daily. Trial 2 and trial 3 horses were offered 1.75 to 2.00 kg DM/100 kg BW in mixed grass hay and 0.2 kg DM/100 kg BW in whole oats daily. The whole oats were fortified to provide trace mineral supplementation. The amount of hay offered was varied with the intention to maintain a body condition score of 5/9. Two different lots of mixed grass hay were used for all three trials. Hay from one lot was designated for the morning feeding (AM) and the other lot was designated for the evening feeding (PM). Each lot of hay was sampled and analyzed for chemical composition at the start of the study. After the initial lot of hay ran out on day 23 of trial 3, a new lot of hay was tested and fed for the AM and PM feedings for the remainder of the trial.

Daily hay and oat rations were split equally and fed at 8:00 am (AM) and 4:00 pm (PM) daily. The AM and PM hay rations were weighed (#ES30R; Ohaus Corp., Parsippany, NJ, USA; 0.1 gram sensitivity) prior to feeding in the AM, and the PM hay allotment was stored in separate Rubbermade® tubs until feeding. The whole oats and corn supplement were weighed prior to feeding using an electronic scale (Catapult 1000; Ohaus Corp., Parsippany, NJ, USA), with 0.01 gram sensitivity, and were stored in labeled plastic bags until feeding. Hay nets were used for messy horses and orts were collected every evening at 10:00 pm and recorded.

### *3.3 c. Digestible Energy Intake*

Digestible energy intake (DEI) was calculated using the digestible energy concentration of the feedstuffs provided by the laboratory chemical analyses from Dairy One (Ithaca, NY, USA) multiplied by the daily amount offered (kg) minus any refusal. The digestible energy of the feed was estimated using the equation developed by Pagan:  $DE \text{ (Kcal/kg dry matter)} = 2,118 + 12.18 (\% \text{ crude protein}) - 9.37 \times (\% \text{ acid detergent fiber}) - 3.83 \times (\% \text{ hemicellulose}) + 47.18 \times (\% \text{ fat}) + 20.35 \times (\% \text{ nonstructural carbohydrate}) - 26.30 (\% \text{ ash})$  (Pagan 1998). Weighed amounts of the three feeds offered were recorded twice daily for each horse over the 42-d trial periods. The DEI was summed per day for 42 d, and the mean DEI for each horse over the 42-d period was compared with the National Research Council (2007) requirement for maintenance.

### *3.3 d. Statistical Analysis*

Horses were grouped according to trial: 12 horses for trial 1, 12 horses for trial 2, and 11 horses for trial 3. Differences in BW and BCS from d-0 to d-42 for each horse were analyzed using paired t-tests (Motulsky 2007). Results of the paired *t* tests were presented as the mean difference with the associated 95% confidence interval. An analysis of variance (ANOVA) model for completely randomized block designs (blocked according to trial) for DEI ( $\text{Kcal} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ ),  $\Delta\text{BW}$  (kg),  $\Delta\text{BCS}$ , and Mcal per kilogram of gain was used to compare differences in the means between trial periods. If the overall ANOVA model was significant, the means were separated using the Tukey-Kramer method of pairwise comparisons (Walker 2002), and the results were represented as

the least squared means  $\pm$  standard error of the mean. Tabular results for the d-0 BWs and BCSs are represented as the mean  $\pm$  SD. Significance was accepted at  $p < 0.05$ .

### 3.4 Results

There were originally 12 horses in trial 3, but one horse was removed because of a chronic hind-limb infection not related to the experiment. All of the other horses remained healthy during the trial periods. The range in initial BW of the horses was 398 kg to 564 kg, with a mean of  $462 \pm 7$  kg ( $n=35$ ). The initial BW and BCS of the horses were not different ( $p=0.21$ ;  $p=0.56$ ) between trial periods (Table 11).

The BW for trial 1 horses ( $n=12$ ) was significantly greater ( $p < 0.001$ ) at the end of the 42 d ( $24 \pm 3$  kg; 95% CI: 18 to 29 kg) than at the beginning; however, the horses did not significantly increase ( $p=0.33$ ) in BCS ( $0.0 \pm 0.5$ ; 95% CI: -0.1 to 0.3; Table 11; Fig. 5a). Likewise, the BW of trial 2 horses ( $n=12$ ) was significantly greater ( $p=0.009$ ) at the end of the 42 days ( $8 \pm 3$  kg; 95% CI: 2 to 14 kg) than at the beginning; however, the horses did not significantly increase ( $p=0.43$ ) in BCS ( $0.5 \pm 0.5$ ; 95% CI: 0.0 to 0.9; Table 11; Fig. 5b). The BW and BCS of horses in trial 3 ( $n=11$ ) was significantly greater ( $p < 0.001$ ;  $p < 0.001$ ) at the end of the 42 days than at the beginning by  $22 \pm 2$  kg (95% CI: 18 to 27 kg) and  $1.0 \pm 0.5$  (95% CI: 0.8 to 1.3), respectively (Table 11, Fig. 5c). Overall, the mean  $\Delta$ BW was  $18 \pm 2$  kg and the horses increased in BCS numerically by  $0.5 \pm 0.5$ .

Change in BW was compared according to trial, and the results are shown in Table 12. The pairwise comparison analysis (Tukey-Kramer) for the difference in the means for the effect of trial on  $\Delta$ BW yielded significantly greater ( $p < 0.001$ ) BW gain of

horses in trials 1 and trial 3 than trial 2. There was no significant ( $p=0.76$ ) difference in  $\Delta BW$  between trial 1 and trial 3 horses (Table 8).

The mean digestible energy intake was significantly different ( $p=0.001$ ) between the three trial periods (Table 12). The combined mean digestible energy intake for the three trials was  $51.73 \pm 3.33 \text{ Kcal} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  ( $23.83 \pm 0.36 \text{ Mcal/d}$ ). Overall, the digestible energy intake exceeded the National Research Council (2007) maintenance requirement by  $60.0 \pm 5.3\%$  (trial 1),  $44.1 \pm 6.3\%$  (trial 2), and  $63.7 \pm 8.4\%$  (trial 3). To arrive at the energy cost of 1 kg of gain, the estimated maintenance requirement was subtracted from the average DE intake to obtain the DE available for gain. Then, the amount of DE available for gain was divided by the average daily gain. The mean Mcal/kg of BW gain required for trials 1 through 3 is shown in Table 12. Although trial 2 horses required nearly twice the Mcal/kg of gain of the other two trials, the results of the ANOVA yielded a trending difference ( $p=0.063$ ) according to trial for Mcal/kg of BW gain.

### 3.5 Discussion

The digestible energy intake was considerably above the NRC (2007) maintenance requirement and therefore explains the overall BW gain in these horses. Although trial 3 horses increased in body condition, there was a minimal  $\Delta BCS$  for the other two trials. The digestible energy intake associated with 1 kg of gain was similar to previous research for trials 1 and 3, with considerably more calories for gain required by trial 2.

The average feeding level and weight gain in the current study was slightly less than the approximate 56 Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> reported over a similar duration (wk 0 to wk 6) in mature Thoroughbred geldings, resulting in 26 kg of weight gain (Suagee et al. 2008). Body weight gain per trial appeared to be associated with the DEI level, i.e., the trial with the highest DEI (trial 1) exhibited the highest amount of weight gain. Within each trial, horses were fed a relatively narrow range of energy intakes, yet there was considerable variation in BW gain between horses. This was especially apparent in trial 1, where horses were fed 52.4 to 54.2 Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> (because all horses were fed at 2.00% of BW in hay), yet BW gain varied considerably from 11 kg to 40 kg total. Possible explanations for this high degree of variation could lie in the horses-horse related differences in basal metabolism and/or the relatively unknown activity. For trials 2 and 3, some of the variation in BW of horses fed hay within the confines of 1.75% to 2.00% of BW in dry matter intake could have come from small adjustments in DEI made by the supervising researcher during the trial period for maintaining a body condition of 5/9.

The energy required per kilogram of gain for trials 1 and 3 was similar to results by Martin-Rosset and Vermorel (1991) for mature Standardbred geldings (18 Mcal DE/kg gain). Horses in trial 2 required considerably greater Mcal DE/kg gain than the other two trials, however, this was not found to be significant in the model (perhaps because of the high degree of horse-horse variation overall). The considerably higher Mcal DE/kg of gain for trial 2 horses could potentially reflect differences with respect to basal metabolism or activity during or prior to the trial period. It was suggested that some of the horses may have had racing careers prior to the trial period and this could

have notably influenced the calories required per kg of gain, particularly if the majority of these conditioned horses were in trial 2. Previous studies have shown that the amount of weight gain in a particular horse is dependent on additional factors, such as the maturity of the horse (Cymbaluk et al. 1989), the composition of the diet (Bush et al. 2001, Martin-Rossett and Vermorel 1991), the body condition of the horse (Webb et al. 1990), basal metabolism (Potter et al. 1987, Stillions and Nelson 1972), and energy expenditure (Graham-Thiers et al. 1991, Pagan and Hintz 1986). An additional consideration is that the maintenance requirement might be elevated in stalled horses exhibiting stereotypical behaviors such as cribbing, weaving or stall-kicking (Luescher et al. 1998). Nevertheless, no significant difference with respect to potentially influential variables, such as age, body condition, or environmental temperature, was found that could be quantified.

The overall minimal change in BCS of the horses can be attributed to the short duration of study and/or the subjective nature of the BCS system, compounded by the use of whole unit scoring. The majority of trial 1 horses gained between 25 kg to 40 kg, however there was no appreciable change in BCS. When horses in trial 2 and 3 gained greater than or equal to 20 kg, this was usually mirrored by a 1 unit increase in BCS. It is possible that the average weight gain was not enough to observe an increase in BCS. A study in Thoroughbred geldings found that 32 kg of BW gain was required per unit increase in BCS (Suragee et al. 2008). In addition, it is possible that the use of whole scoring units limited the sensitivity of the measurement, especially because body condition scoring is subjective in nature. Furthermore, the study may have been too short to observe a change in BCS. Suragee and colleagues (2008) found that when BCS

was regressed against time, one unit increase in BCS required 80 days in Thoroughbred geldings fed above maintenance energy intake. Studies showing weight loss in horses have found that short-term weight-loss management rarely achieves an appreciable change in body condition – particularly because visceral tissue is depleted more rapidly than subcutaneous tissue (Argo et al. 2012, Dugdale et al. 2010). Likewise, under-conditioned horses may first replenish visceral stores before covering the external skeleton. In this manner, body condition scoring may not be truly representative of weight gain, especially in a short duration study.

Limitations in this study include not being able to measure the energy digestibility of the feedstuffs by a digestibility trial prior to the start of this study and not being able to assess differences in energy expenditure between horses. In addition, it is possible that hay waste may have occurred through the removal during stall cleaning or from the open stall design. It was noted that on windy days, some hay blew out of the stalls or into adjacent stalls.

Digestible energy intake above maintenance that was associated with 1 kilogram of BW gain was similar to previous reports (Martin-Rosset and Vermorel 1991, NRC 2007). The variation in weight gain may have been dependent upon additional factors that were not quantified in this study. Since horses in this study were around the same age and the composition of the diet remained fairly constant, possible variation in BW gain between horses most likely arose from differences in basal metabolism and energy expenditure during the trial period.

Table 8. Nutrient Analysis for the Whole Oats and Mixed Grass Hay for Trial 1 (Dry Matter Basis)

|                             | Whole oats | Mixed grass hay |      |
|-----------------------------|------------|-----------------|------|
| Items                       |            | AM              | PM   |
| Crude protein (%)           | 10.9       | 12.4            | 14.1 |
| Acid detergent fiber (%)    | 14.4       | 32.2            | 33.2 |
| Neutral detergent fiber (%) | 27.0       | 51.6            | 50.6 |
| Fat (%)                     | 5.6        | 3.5             | 3.8  |
| Ash (%)                     | 4.4        | 6.5             | 6.8  |
| Calcium (%)                 | .18        | .76             | .96  |
| Phosphorus (%)              | .42        | .34             | .33  |
| Digestible energy (Mcal/kg) | 3.3        | 2.4             | 2.4  |

Table 9. Nutrient Analysis for the Whole Oats and Mixed Grass Hay for Trial 2 (Dry Matter Basis)

|                             | Whole oats | Mixed grass hay |      |
|-----------------------------|------------|-----------------|------|
| Items                       |            | AM              | PM   |
| Crude protein (%)           | 10.3       | 12.4            | 14.1 |
| Acid detergent fiber (%)    | 16.0       | 32.2            | 33.2 |
| Neutral detergent fiber (%) | 30.5       | 51.6            | 50.6 |
| Fat (%)                     | 5.6        | 3.5             | 3.8  |
| Ash (%)                     | 4.4        | 6.5             | 6.8  |
| Calcium (%)                 | .11        | .76             | .96  |
| Phosphorus (%)              | .38        | .34             | .33  |
| Digestible energy (Mcal/kg) | 3.3        | 2.4             | 2.4  |

Table 10. Nutrient Analysis for the Whole Oats and Mixed Grass Hay for Trial 3 (Dry Matter Basis)\*

|                             | Whole oats | Mixed grass hay |      |
|-----------------------------|------------|-----------------|------|
| Items                       |            | AM              | PM   |
| Crude protein (%)           | 11.2       | 12.4            | 14.1 |
| Acid detergent fiber (%)    | 11.7       | 32.2            | 33.2 |
| Neutral detergent fiber (%) | 22.0       | 51.6            | 50.6 |
| Fat (%)                     | 5.6        | 3.5             | 3.8  |
| Ash (%)                     | 4.4        | 6.5             | 6.8  |
| Calcium (%)                 | .15        | .76             | .96  |
| Phosphorus (%)              | .37        | .34             | .33  |
| Digestible energy (Mcal/kg) | 3.3        | 2.4             | 2.4  |

\*After day 23, horses we fed a new hay composed of 14.9% crude protein, 36.6% acid detergent fiber, 61.8% neutral detergent fiber, 0.31% calcium, 0.31% phosphorus, and 2.05 digestible energy (Mcal/kg)

Table 11. Initial and Final Body Weight (BW) and Body Condition Score (BCS)

|                    | Trial*    |           |           |
|--------------------|-----------|-----------|-----------|
| Items              | 1         | 2         | 3         |
| Initial<br>BW (kg) | 447 ± 23  | 476 ± 49  | 462 ± 39  |
| BCS                | 4.5 ± 1.0 | 4.5 ± 1.0 | 4.0 ± 1.0 |
| Final              |           |           |           |
| BW (kg)            | 471 ± 26  | 484 ± 46  | 485 ± 42  |
| BCS                | 4.5 ± 0.5 | 5.0 ± 1.0 | 5.5 ± 0.5 |

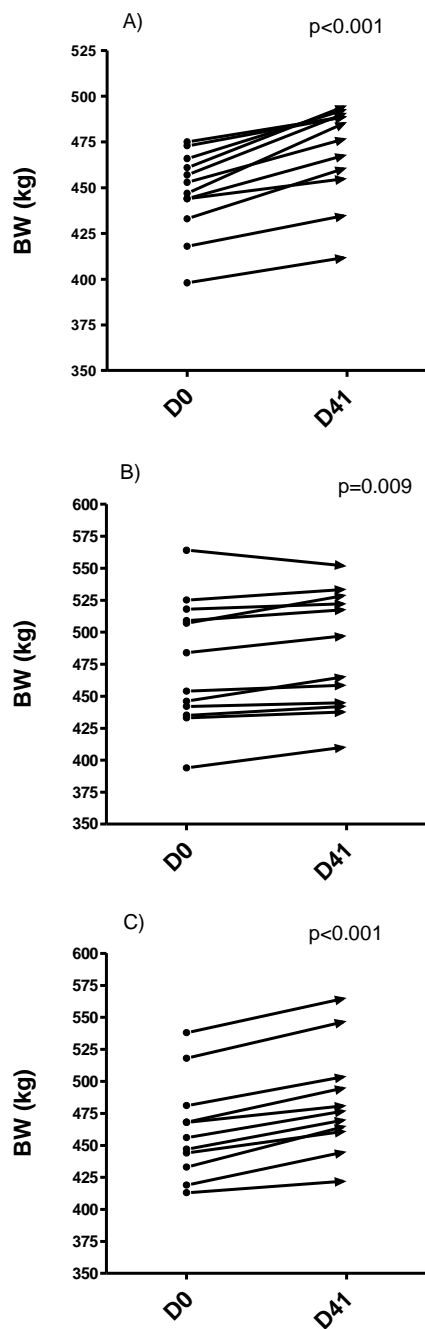
\*Trial: 1 = (n=12); 2 = (n=12); 3 = (n=11)

Table 12. Digestible Energy Intake (DEI), Change in body weight ( $\Delta$ BW), and Digestible energy (Mcal)/kg of gain in Horses

|  | Trial†                    |                           |                           |
|--|---------------------------|---------------------------|---------------------------|
| Items  | 1                         | 2                         | 3                         |
| DEI (Kcal·kg BW <sup>-1</sup> ·d <sup>-1</sup> ) | 53.21 ± 0.58 <sup>a</sup> | 47.68 ± 2.10 <sup>b</sup> | 51.22 ± 2.19 <sup>c</sup> |
| $\Delta$ BW (kg)                                 | 24 ± 3 <sup>a</sup>       | 8 ± 3 <sup>b</sup>        | 22 ± 2 <sup>a</sup>       |
| Digestible energy (Mcal/kg BW gain)              | 18.98 ± 2.29              | 34.07 ± 8.22              | 17.97 ± 2.43              |

\*Means values within a row with different subscripts differ (p<0.05).

†Trial: 1 = (n=12); 2 = (n=12); 3 = (n=11).



**Figure 5.** Within-horse change in body weight from d-0 to d-41 for (a) trial 1, (b) trial 2 and (c) trial

## Literature Cited

Argo, C. M., G. C. Curtis, D. Grove-White, A. H. A. Dugdale, C. F. Barfoot, P. A. Harris. 2012. Weight loss resistance: A further consideration for the nutritional management of obese. *Equine Vet. J.* 194(2): 179-188. DOI:10.1016/j.tvjl.2012.09.020

Bush, J. A., D. E. Freeman, K. H. Kline, H. R. Merchen, G. C. Fahey. 2001. Dietary fat supplementation effects on in vitro nutrient disappearance and in vitro nutrient intake and total digestibility by horses. *J. Anim. Sci.* 79(1): 232-239. DOI: 2001.791232x

Carter, R. A., L. J. McCutcheon, L. A. George, T. L. Smith, N. Frank, R. J. Geor. 2009. Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses. *Am. J. Vet. Res.* 70(10): 1250-1258. DOI: 10.2460/ajvr.70.10.1250

Cymbaluk, N. F., G. I. Christison, D. H. Leach. 1989. Nutrient utilization by limit- and ad-libitum-fed growing horses. *J. Anim. Sci.* 67(2): 414-425. DOI:10.2134/jas1989.672414x

Dugdale, A. H. A., G. C. Curtis, P. Cripps, P. A. Harris, C. M. Argo. 2010. Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Vet. J.* 42(7): 600-610. DOI:10.1111/j.2042-3306.2010.00110.x

Geor, R. J. 2008. Metabolic predispositions to laminitis in horses and ponies: Obesity, insulin resistance and metabolic syndromes. *J. Equine Vet. Sci.* 28(12): 753-759. DOI: 10.1016/j.jevs.2008.10.016

Geor, R. J., P. Harris. 2009. Dietary management of obesity and insulin resistance: Countering risk for laminitis. *Vet. Clin. N. Am. Equine Prac.* 25(1): 51-65. DOI: 10.1016/j.cveq.2009.02.001

Gill, J. C., S. E. Pratt-Phillips, R. Mansmann, P. D. Siciliano. 2016. Weight loss management in client-owned horses. *J. Equine Vet. Sci.* 39(2016): 80-89. DOI: 10.1016/j.jevs.2015.12.014

Graham-Thiers, P., D. S. Kronfeld, K. A. Kline. 1999. Dietary protein influences acid base responses to repeated sprints. *Equine Vet. J. Suppl.* 33: 463. DOI:10.1111/j.2042-3306.1999.tb05266.x

Henneke, D., G. Potter, J. Kreider, B. Yates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15(4): 371-372. DOI: 10.1111/j.2042-3306.1983.tb01826.x

Heusner, G. 1993. Ad libitum feeding of mature horses to achieve rapid weight gain. *Proc. 13<sup>th</sup> Equ. Nut. Phys. Soc. Symp.* Gainesville, FL. 86.

Kronfeld, D. S. 1993. Starvation and malnutrition of horses: Recognition and treatment. *J. Equine Vet. Sci.* 13(5): 298-304.

Lindåse, S. S., K. E. Nostell, C. E. Müller, M. Jensen-Waern, J. T. Bröjer. 2016. Effects of diet-induced weight gain and turnout to pasture on insulin sensitivity in moderately insulin resistant horses. *Am. J. Vet. Res.* 77(3): 300-309. DOI:10.2460/ajvr.77.3.300

Luescher, U. A., D. B. McKeown, H. A. Dean. 1998. Cross-sectional study on compulsive behavior (stable vices) in horses. *Equine Vet. J. Suppl.* 27: 14-18.

Martin-Rosset, W., M. Vermorel. 1991. Maintenance energy requirement variation determined by indirect calorimetry and feeding trials in light horses. *J. Equine Vet. Sci.* 11(1): 42-45. DOI: 10.1016/S0737-0806(06)81246-3

Motulsky, H. J. 2007. Prism 5 Statistics Guide. GraphPad Software Inc., San Diego, CA.

Munoz, A., C. Riber, P. Trigo, F. Castejon. 2010: Hematology and clinical pathology data in chronically starved horses. *J. Equine Vet. Sci.* 30(10): 581-589.

National Research Council. 2007. Nutrient Requirements of Horses. 6th revised ed. National Academy Press, Washington, DC.

Pagan, J. D. 1998. Measuring the digestible energy content of horse feeds. In: Pagan, J. D. (ed.), *Advances in Equine Nutrition*. Nottingham University Press, Nottingham, UK. 71-76.

Pagan, J. D., H. F. Hintz. 1986. Equine Energetics II. Energy expenditure in horses during submaximal exercise. *J. Anim. Sci.* 63(3): 822-830. DOI:10.2134/jas1986.633822x

Potter, G. D., G. W. Webb, J. W. Evans, S. P. Webb. 1987. Digestible energy requirements of Belgian and Percheron horses. *Proc. 10<sup>th</sup> Equ. Nut. Phys. Soc. Symp.* Ft. Collins, CO. 133-138.

Potter, G. D., S. P. Webb, J. W. Evans, G. W. Webb. 1990. Digestible energy requirement for work and maintenance of horses fed conventional and fat-supplemented diets. *J. Equine Vet. Sci.* 10(3): 214-218. DOI:10.1016/S0737-0806(06)80163-2

Stillions, M. C., W. E. Nelson. 1972. Digestible energy during maintenance of light horses. *J. Anim. Sci.* 34(6): 981-982. DOI: 10.2134/jas1972.346981x

Stull, C. L. 2009: Refeeding the starved horse. In: Conference Sponsors. 111.

Suragee, J. K., A. O. Burk, R. W. Quinn, E. D. Petersen, T. G. Hertsock, L. W. Douglas. 2008. Effect of diet and weight gain on body condition scoring in Thoroughbred geldings. *J. Equine Vet. Sci.* 28(3): 156-166. DOI:10.1016/j.jevs.2008.01.019

Suagee, J. K., B. A. Corl, M. V. Crisman, R. S. Pleasant, C. D. Thatcher, R. J. Geor. 2013. Relationships between body condition score and plasma inflammatory cytokines, insulin, and lipids in a mixed population of light-breed horses. *J. Vet. Int. Med.* 27(1): 157-163.

Walker, G. A. 2002. *Common Statistical Methods for Clinical Research*, 2nd vol. SAS Institute, Cary, NC.

Webb, S. P., G. D. Potter, J. W. Evans, G. W. Webb. 1990. Influence of body fat content on digestible energy requirements of exercising horses in temperate and hot environments. *J. Equine Vet. Sci.* 10(2): 116-120. DOI:10.1016/S0737-0806(06)80118-8

Witham, C. L., C. L. Stull. 1998. Metabolic responses of chronically starved horses to refeeding with three isoenergetic diets. *J. Am. Vet. Med. Assoc.* 212(1998): 691-696.

## CHAPTER IV: EXPERIMENT 3

### Activity level and digestible energy intake in horses

#### 4.1 Abstract

This preliminary study was conducted to quantify the individual variation in activity (heart rate (HR) and distance traveled (DT)), and dry matter intake (DMI) in horses. Eight mature, idle geldings (mean  $\pm$  standard deviation; BW  $618 \pm 58$  kg; 5-12 years; BCS  $7.0 \pm 1.5$ ; Quarter Horses) were maintained continuously on a  $210 \times 150$  m<sup>2</sup> tall-fescue pasture for 12 days. Horses were alternated through two, 3-d collection periods of HR /DT assessment by Polar V800 activity monitoring devices (Polar Electro, NY) and total fecal collection by equine nappies. Feces were weighed every 4 h and pooled daily. Daily fecal (1 per horse) and pasture (1 per day) samples were analyzed for acid insoluble ash (AIA), and the dry matter digestibility was calculated as  $(1 - \text{AIA}_{\text{diet}} / \text{AIA}_{\text{feces}}) \times 100$ . Estimating DMI was unsuccessful because the AIA measurement in the feces showed considerable within-horse variation. The repeatability for HR, DT, and fecal output (FO) between horses was calculated as the within-horse standard deviation ( $S_w$ ), and the product of  $S_w$  and 2.77 was the repeatability coefficient. The coefficient of variation (CV) was used to determine the within-horse repeatability. One-way analysis of variance included only the significant model parameters horse and group to validate differences between horses. Heart rate and FO were repeatable measures between Group 1 and Group 2 horses ( $\pm 4$  bpm and  $\pm 1.63$  and  $1.66$  g  $\cdot$  kg BW<sup>-1</sup>  $\cdot$  d<sup>-1</sup>, respectively). The mean CV for Group 1 and Group 2 horses were 3.6% and 3.3% (HR), 13.1% and 22.8% (DT), and 7.0% and 6.6% (FO), respectively. The results suggest the following: within-horse differences in daily HR were small and the significant ( $p < 0.001$ )

differences in HR between horses may be attributed to differences in metabolism and voluntary activity; daily DT is an extremely variable measure ( $p < 0.001$ ) within- and between-horses meaning that horses were not consistent in their grazing behavior across days of study; and FO exhibited little within-horse variation, but was significantly ( $p < 0.001$ ) different between horses, suggesting that horses may have consumed different levels of intake.

## 4.2 Introduction

When the dry matter availability is not limiting, pasture can meet or exceed overall energy and protein requirements for most physiological classes (Dowler et al. 2012, NRC 2007). Specifically, mature idle horses are most at risk for consuming calorie intakes in excess of requirements, leading to obesity. Based on previous methods, pasture intake in continuously grazing horses spans a large range (1.5% to 3.1% BW in DM; NRC 2007); however, there is limited research quantifying voluntary intake from pasture or the difference in dry matter intake (DMI) between horses. There is some evidence that the indigestibility markers, including n-alkanes (Chavez et al. 2014), chromic oxide (Haeleyn et al. 1966, Olsson and Ruudvere 1955), and acid insoluble ash (AIA; Cuddeford and Hughes 1990, Miraglia et al. 1999), can be used to quantify DMI in horses (Bergero et al. 2004, 2009, Sutton et al. 1977). The internal marker, AIA has been applied to measure the digestibility of hay and concentrate diets in horses with some success. Based on alternative methods, the dry matter digestibility from grass pasture ranges from 53 to 61% (Menard et al. 2002, Stevens et al. 2002, 2007, Chavez et al. 2014) and varies based on the plant species, season, and between animals.

Evaluating the voluntary activity of horses may provide insight into activity-related factors that predispose a horse to obesity. The NRC (2007) estimates that the maintenance digestible energy (DE) requirement is subject to variability ( $\pm$  approximately 10%), that is dependent upon horse temperament (calm versus attentive) and husbandry conditions (stall confinement versus turnout) of the horse. There is limited knowledge concerning the energy expenditure value associated with continuous pasture grazing. Because of the strong relationship between oxygen utilization and HR, energy expenditure above maintenance can be estimated in exercising horses when the average heart rate (HR) and intake rate is known (NRC 2007). A model using HR in grazing horses could be developed if the within-horse variation of average daily HR was relatively small and the energy expended above maintenance requirements could be quantified. Therefore, the objective of this study was to present novel findings quantifying the individual variation in DMI and activity between horses at pasture. Assessing activity and pasture intake variability may provide insight into identifying better ways to manage horses for the maintenance of body condition

#### 4.3. Materials and Methods

##### *4.3 a. Animals and Animal Care*

The following experimental protocol was approved by the North Carolina State University Institutional Animal Care and Use committee (No. 15-134T). The experiment was conducted from June 8, 2015 to June 20, 2015 at the North Carolina State University Equine Research Unit in Raleigh, North Carolina.

Eight mature, idle, Quarter Horse geldings (mean  $\pm$  standard deviation; BW 618  $\pm$  59 kg; Age 5 to 12 years; BCS 7.0  $\pm$  1.5) were maintained continuously on a 210  $\times$  150 m<sup>2</sup> (7.7 acre) pasture for 17 days, which included a 5 day adaptation period prior to the start of the study. The pasture was in a vegetative state and consisted of primarily nontoxic, endophyte-infected tall fescue (*Lolium arundinaceum* Schreb cv Max-Q, Pennington Seed, Madison, GA). The pasture contained herbage available for grazing in excess of the calories required to maintain body weight (1,300 kg DM/acre). Horses received pasture as their sole diet. Horses had access to two, large rubber water tubs in the lower north corner of the pasture. Scale body weight (BW) was recorded at 7:00 am on d 0, d 3, d 6, d 9, and d 12. Body weights were measured using an electronic large animal scale with  $\pm$  1.0 kg sensitivity (Smart Scale 200, Gallagher Animal Management, USA). Body condition scores were determined according to the 1 to 9 point scale, modified using 1/2 units (Henneke et al. 1983). Horses that were wearing equine nappies were treated daily with fly repellent and hosed down on days that were 32 °C or greater.

#### *4.3 b. Experimental Design*

Because the number of activity monitors and equine nappies was limited to four, the horses were randomly assigned to Group 1 (n=4) or Group 2 (n=4) and alternated through two, 3-d periods of activity tracking (distance traveled and heart rate monitoring) and fecal collection (Table 13).

#### *4.3 c. Fecal Collection and Sampling*

Feces were collected over two, 3-d periods of continuous grazing periods using equine nappies (equine nappies, Equisan Marketing Pty Ltd., Australia). Six times daily

(every 4 hours), the feces was weighted, mixed, and a 10% sample was collected and refrigerated. The six, 10% samples of feces from each horse were pooled, mixed, and a single daily fecal sample (100 g) for each horse was collected and stored for later analysis in a -20 °C freezer. Pasture samples were collected once daily at 2:30 PM and stored in a -20 °C freezer. Forage and feces samples were analyzed by Dairy One (Ithaca, NY) for dry matter (DM) and acid insoluble ash (AIA) testing according to Van Keulen and Young (1977) and Miraglia et al. (1999) for cumulative samples of daily pasture and feces. Dry matter digestibility (DMD) was calculated using the following equation (Bergero et al. 2009):

$$\text{DMD (\%)} = (1 - \text{AIA}_{\text{diet}} / \text{AIA}_{\text{feces}}) \times 100$$

where  $\text{AIA}_{\text{diet}}$  is the AIA content of the pasture on a DM basis in g/kg and  $\text{AIA}_{\text{feces}}$  is the AIA content of the feces on a DM basis in g/kg. The digestibility was calculated as 1-indigestible fraction. The dry matter intake (DMI; g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>) was calculated according to Peiretti et al. (2006):

$$\text{DMI} = \text{Fecal output} \times (1 - \text{DMD})^{-1}$$

Daily fecal output (FO) was expressed as kg per day and g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>. The FO in g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> was calculated by multiplying the kg DM of the feces by 1000 and dividing by BW.

#### *4.3 d. Distance Traveled and Heart Rate Monitoring*

Distance traveled (DT) and HR measurements were obtained through digital recordings on Polar Equine V800 activity monitors (Polar Electro Inc., Lake Success, NY, USA) over two, 3-d periods of continuous grazing. The HR electrode band was secured around the girth circumference of the horse and pinned in place. The receiver was

secured with vet wrap to the top of the belt, synced and started. Water and ultrasound gel were continuously applied to the electrode region to maintain conductivity. The receivers were checked at 2:00 pm (EST) daily to insure they were properly recording. The receivers were stopped and removed from the horses at 7:00 am (EST) and 7:00 pm daily. At those times, the daytime (8:30 am to 7:00 pm) or nighttime (8:30 pm to 7:00 am) activity was logged, the data was downloaded to the software program (PolarFlow.com), and the receivers were charged. Data collected from the devices included: date, time, day of study, period of feed (daytime or nighttime), receiver feed (normal, low battery, paused, or not running), position of device (normal or shifted), start-time, end-time, duration of feed (hr), distance traveled (km), average heart rate (bpm), temperature, weather, and mood of the horse. Collecting the data and charging the devices took approximately 1½ hours, and then the receivers were put back on the horses and re-started. The Polar V800 error rate for distance was 0.26% and 1.96% for trueness and accuracy, respectively (fellrnr.com).

For each horse, the average daytime and nighttime collection periods of HR were averaged to yield a mean daily HR (bpm). The total daytime and nighttime distance traveled data was summed to yield total daily DT (km/d).

#### *4.3 e. Statistical Analysis*

Differences between horses in HR, DT, and FO were assessed according to a one-way analysis of variance (ANOVA) with the model parameters Group and horse. Day of collection and period of collection were not included in the model because of insignificance. Daily heart rate, DT, and FO values are presented as the least squared mean and the 95% CI. The coefficient of variation (CV) for serial HR, DT, and FO

measurements were calculated as the standard deviation divided by the mean. The within-horse standard deviation ( $S_w$ ) was calculated as the square root of the residual mean square error. The repeatability coefficient (RC) was calculated as the product of  $S_w$  and 2.77 (Bland and Altman 1996). For repeatable measurements, the difference between paired measurements on the same subjects was expected to be less than the RC value 95% of the time. A p-value of  $< 0.05$  was considered significant.

#### 4.4 Results

##### *4.4 a. Dry Matter Digestibility and Fecal Output*

Table 14 shows the AIA composition of the feed and feces, the dry matter indigestibility and digestibility, and the estimated DMI for three of the eight horses. There was considerable within-horse variation noted in the  $AIA_{feces}$  measurement. Thus, the AIA method for measuring DMD from grazing horses was unsuccessful in this study.

Fecal collection was successfully carried out over 6 days for each horse, so the variability in FO measures was compared between horses. There was a significant ( $p < 0.001$ ) difference in FO between horses. The CV for FO for Group 1 and Group 2 horses was 7.03% and 6.61%, respectively, meaning that there was little variation within-horses. The RC values for Group 1 and Group 2 horses were 1.63 g DM/kg BW and 1.66 g DM/kg BW, respectively (*i.e.*, 95% of the differences between pairs of samples from the same horse were expected to be less than this amount). According to Fig. 8, three horses (horse 2, 6, and 7) exhibited values that differed more than the RC by 33%, 33%, and 23%, respectively (67%, 67%, and 87% of the variation could be

explained by the model). Overall, the mean daily FO was  $8.44 \pm 1.67$  g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>, ranging from 7.43 to 12.13 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> (Table 15).

#### *4.4 b. Heart Rate Monitoring and Distance Traveled*

There was significant ( $p < 0.001$ ;  $p < 0.001$ ) horse to horse variation in HR and DT. The mean HR of Group 1 was significantly ( $p < 0.001$ ) greater than Group 2. The mean DT of Group 1 was significantly ( $p < 0.001$ ) lower than Group 2. The CVs for HR and DT for Group 1 and Group 2 were 3.59% and 3.26% and 13.14% and 22.79%, respectively. The RC for both groups was 4 bpm for HR and 0.67 km (Group 1) and 1.58 km (Group 2) for DT (Figs. 6 and 7). For HR, only two horses (horse 1 and 6) exhibited values that differed more than the RC by 27%. For DT, three horses (horse 4, 5, and 8) exhibited values that differed more than the RC by 20%, 27%, and 7%, respectively. Mean HR was 42 bpm and daily DT averaged 2.19 km/d (Table 16 and 17).

#### 4.5 Discussion

The results of this study showed that there was significant variation between horses in activity and possibly differences in pasture intake between horses. The within-horse variability (according to CV and RC values) was generally low for HR and FO, meaning good method performance. Individual variation in activity was successfully quantified through the use of Polar Equine V800 activity monitoring devices. Although the AIA determination of DMI was unsuccessful, differences in FO between horses may indicate variability in DMI.

This study presents novel finding of pasture digestibility that was estimated according to AIA. Several studies have successfully measured AIA as an indicator of the

digestibility of hay and concentrate diets (Bergero et al. 2009, Sutton et al. 1977, Orton et al. 1985, Cuddeford and Hughes 1990). Thonney et al. (1985) states that the AIA content of the diet should exceed 7.5 g/kg DM, otherwise the analytical precision of the test for AIA declines. In comparison with the AIA method performed using hay-based diets, the AIA concentrations in pasture and feces were considerably lower. The pasture AIA content ranged from 7.5 g/kg DM to 10.4 g/kg DM, whereas the AIA content of Brome hay and Prairie hay were 12 g/kg DM and 45 g/kg of DM according to Van Keulen and Young's (1997) methods. Although diurnal and daily variations in fecal AIA content have been minimal in previous study, this was not the case in the present study. There was considerable within-horse variation that occurred between daily samples, and the digestibility estimates obtained were outside of the expected range for the quality of pasture that was grazed. Since the AIA fraction in the feces is a small number, the potential for error has to be carefully controlled for in the methods and preparation. Possible inconsistencies during mixing and sampling, despite researchers following strict protocols, could have resulted in some error. Sales and Janssens (2003) reported that inconsistencies in the recovery rate of AIA were associated with soil ingestion by the animals, soil or dust in the diet or feces, and feed sampling that did not accurately represent the diet. Horses were observed consuming dirt while at pasture, which could have significantly impacted the fecal AIA measurement. It appeared that small changes in AIA concentration in the feces exhibited the greatest influence on DMD, since this parameter was located in the denominator of the equation. Consequently, it was determined that the AIA marker method was not reliable for use in grazing horses, according to the results of this study.

Dry matter digestibility of pasture has been previously measured with success using n-alkanes. Stevens and colleagues (2002) found that Ryegrass and Kikuyu pastures yielded DMDs of 53.5% and 58.6%, respectively. Using the same tall-fescue pasture as the current study, Chavez et al. (2014) estimated the DMD as  $58.6 \pm 2.9\%$  with C31 and C33 alkane markers, having been validated within-trial by feeding and measuring the digestibility of a hay-based diet. When a range of 55% and 60% DMD was applied, the estimated DMI from the current study would have been between  $18.75 \pm 3.71 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  ( $11.37 \pm 2.11 \text{ kg/d}$ ) and  $21.10 \pm 4.18 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  ( $12.79 \pm 2.37 \text{ kg/d}$ ), respectively (Table 23), which was within the acceptable range that has been reported in the literature (15 to 32 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>; mean of 20 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>; Chavez et al. 2014, Dulphy et al. 1997, Feurance et al. 2001, Marlow et al. 1983, NRC 2007). Based on the proposed DMI range, the DEI would have been between  $42.2 \pm 8.4 \text{ Kcal} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  and  $47.5 \pm 9.4 \text{ Kcal} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ , respectively (Table 24). This estimation was similar to the 47 Kcal·kg BW<sup>-1</sup>·d<sup>-1</sup> in digestible energy intake from Chavez et al. (2014) when using the North Carolina State University Research Unit pastures and some of the same horses that were used in the present study.

The RC measures the repeatability of measures for the evaluation of method performance. The RC of FO was low, representing approximately 10% of the mean. A reasonable expectation is that the difference between two samples from the same subject 95% of the time would be less than 1 kg DM of difference. In this case, the actual RC value of 0.82 kg was less than this proposed cut-off. The within-horse FO differed more than the RC for three of the eight animals. A possible explanation for the variability in daily FO was most likely related to errors that occurred with the collection

of feces. Two of these horses were obese (BCS>8/9); therefore, the collection harnesses had to be modified to adapt to their large sizes. Bailing twine was used to create tail ties and metal carabiner climbing clips were used to secure the side straps on the collection bag to the blanket portion of the harness to prevent breakage. Despite these modifications, the researchers experienced difficulty in keeping the harnesses from shifting out of position and breakage of the fecal collection harnesses was a common occurrence. Although researchers used much diligence to make sure that no feces was lost, the researchers kept detailed records of when the gear was broken and feces could have been lost. The development of a more durable collection harnesses is warranted, with the incorporation of a surcingle and harness straps.

The mean FO was similar to results by Chavez et al. (2014) in horses allowed to graze for 18 h per day on tall-fescue pasture ( $9.5 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  to  $11.7 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ ) and Holland et al. (1998) for continuously grazing horses on mixed-grass pastures ( $6.13 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  and  $9.04 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ ). Despite low within-horse and between group variations in FO, the ANOVA analysis showed significant between-horse differences in FO, even when horse 8 was excluded from the model. Because of the positive relationship between FO and DMI (Peiretti et al. 2006), horses may have potentially consumed different levels of intake. Additionally, it is noteworthy to mention that FO was positively-correlated with BW, which was represented by the equation:  $y = 5.0815x + 441.71$ , where y is BW (kg) and x is the average daily FO (kg) ( $R^2 = 0.40$ ). The initial body weight of the horses ranged from 531 kg to 691 kg. Fecal collection from horse 8 was reportedly the most comprehensive; the large difference in

FO in this horse could have come from inefficiency of digestibility or a high rate of intake.

Heart rate was well reported using the Polar V800 HR devices, as indicated by the data feeds from the Polar Flow software program. Because basal HR was computed as the HR recorded by the device every second and the data feeds were long, device-related problems with the collection feed minimally affected the overall average (as long as errors were minimal). Previous literature suggests that resting HR between horses ranges from 25 to 40 bpm (Hodgson et al. 2014). Overall, mean daily HR was similar to that reported by Gehreke et al. (2011) in grazing horses (43 bpm). Within-horse variations in HR were generally minimal and were not greatly influenced by day. The RC of 4 bpm represented approximately 10% of the mean, but could have been slightly inflated from horse 1, 2, and 6. Two horses exhibited HRs that differed more than the RC value. Variation in these horses appeared to be related to differences between the 3-d periods of monitoring. Horse 1 had an overall higher HR during the first 3-d period (45, 49, and 42 bpm) than the second period (41 bpm). It is possible that this horse exhibited some excitement, possibly associated with wearing the device the first two days of the study, but adjusted thereafter. The opposite was the case for horse 6. This difference in HR could have been related to temperature. The daytime temperature was 2 °C greater during the second 3-d period of monitoring and possibly affected this gelding since he was the oldest and most dominant horse. There were significant differences with respect to Group and horse on HR. No logical explanation for the effect of Group on HR could be found, but horse-related differences in HR could be explained by differences in metabolism and activity that have been previously

reported in the literature (NRC 2007). Differences in basal metabolism and pasture-associated activities, including preference for grazing, meandering, and social play could significantly affect daily HR between horses. Herd social status may also play a significant role in activity and HR level, although this was not quantified in the current study (Boyd 1991, Ellard and Crowell-Davis 1989, NRC 2007).

An additional noteworthy finding is that the horse with the highest mean HR was the most obese horse (BCS 9/9). The occurrence of heat-stress in obese horses has been previously mentioned (Cymbaluk and Christison 1990, Quinn et al. 2006, Webb et al. 1989). Webb and colleagues (1989) found that horses with a BCS of 7.5 had a harder time dissipating heat in hot weather (temperature 32 °C and 44% humidity) than horses with a BCS of 5. Similarly, Geor and colleagues (1995) found that throughout exercise and recovery, horses in hot and humid (32-34°C, 80-85 relative humidity) conditions had higher HRs compared with horses in milder conditions. Therefore, the high temperatures towards the end of this study could have contributed to an elevation in HR; especially for the overweight, fat-insulated horses because of the effect heat-stress has on cardiovascular and respiratory rates.

Distance traveled data collected from day 1 was removed from the data set because horses traveled 2-fold farther on day 1 than the other days, which resulted from horses with monitors following the horses with nappies to the gate when they were lead to and from the pasture every 4 hours for fecal collection. After day 1, the horses wearing the monitors adjusted to this activity and did not follow the other horses. Horses were not consistent in grazing activity across days of study. The within- and between horse repeatability for DT was high. According to the repeatability of the

other measures (e.g., heart rate and fecal output), the RC for DT should have been 0.22 km (equal to 10% of the mean). The RC for Group 1 horses represented approximately 30% of the mean, whereas the RC from Group 2 horses represented 72% of the mean. Additionally, the CV for Group 2 were outside of an acceptable range for showing good method performance (>20%). Within-horse distance traveled differed more than the RC in three of the eight animals. One of the horses (horse 5) traveled farther distances on the last two days of the study because of aggravation reported in response to wearing the Polar V800 activity monitoring device. For horse 4, the variability in measures was related to problems with collection because of device error. Horse 3 traveled the least distance per day because of a musculoskeletal disorder in this gelding's left hind limb, causing a pronounced limp. There was a significant effect of Group on distance traveled, which could be explained by horse 3 residing in Group 1 and horse 5 residing in Group 2. Because of the small sample size, these horses could have notably affected the Group means. Additional variability in DT between horses may arise from factors such as the weather (a record high temperature was recorded on Day 9; 38 °C, humidity of 84%, and a heat index of 40 °C), horse temperament, herd dynamics, physical fitness, *etc.* (NRC 2007). Hierarchy among horses has been well documented (Boyd 1991, Ellard and Crowell-Davis 1989, NRC 2007) and may affect feeding or drinking behavior in horses from competition for resources. It is possible that this day to day variance in DT could be reduced by averaging more days of collection in future study.

Although previous studies have reported grazing horses traveling farther distances, the results from this study is similar to the 2.66 km/d reported by Shingu and colleagues (2000). It would seem intuitive that the herbage mass availability would

significantly impact foraging activities. Therefore, horses on lush pasture would require less travel to consume their daily requirement than horses on sparse pasture. In addition, it is possible that confounding variables, such as the extreme heat and water placement, lessened the desire and motivation for the horses to travel farther distances.

Assessing activity and pasture intake variability may provide insight into identifying better ways to manage horses for maintaining zero energy balance. In addition, the repeatability of model parameters is important information for determining the effectiveness of the Polar V800 devices when evaluating pasture-associated activity in horses. Overall, HR and FO were repeatable measures, although there was some between-horse variability which might suggest uniqueness in metabolism and DMI in horse. Differences in activity ultimately affect energy balance; heart rate showed considerable uniqueness among horses which was possibly associated with differences in metabolism and activity. Heart rate may be a good predictor for modeling energy expenditure in grazing horses and warrants further study using a larger sample size.

Table 13. Experimental Design

|       | Group 1             |   |   |   | Group 2             |   |   |   |
|-------|---------------------|---|---|---|---------------------|---|---|---|
| Horse | 1                   | 2 | 3 | 4 | 5                   | 6 | 7 | 8 |
| Day   |                     |   |   |   |                     |   |   |   |
| 1     | Fecal collection    |   |   |   | Activity monitoring |   |   |   |
| 2     | Fecal collection    |   |   |   | Activity monitoring |   |   |   |
| 3     | Fecal collection    |   |   |   | Activity monitoring |   |   |   |
| 4     | Activity monitoring |   |   |   | Fecal collection    |   |   |   |
| 5     | Activity monitoring |   |   |   | Fecal collection    |   |   |   |
| 6     | Activity monitoring |   |   |   | Fecal collection    |   |   |   |
| 7     | Fecal collection    |   |   |   | Activity monitoring |   |   |   |
| 8     | Fecal collection    |   |   |   | Activity monitoring |   |   |   |
| 9     | Fecal collection    |   |   |   | Activity monitoring |   |   |   |
| 10    | Activity monitoring |   |   |   | Fecal collection    |   |   |   |
| 11    | Activity monitoring |   |   |   | Fecal collection    |   |   |   |
| 12    | Activity monitoring |   |   |   | Fecal collection    |   |   |   |

Table 14. Acid Insoluble Ash Measurements from Pasture and Fecal Samples in Grazing Horses

| Horse     | Sample No. | AIA <sub>Feed</sub> (g/kg) | AIA <sub>Feces</sub> (g/kg) | Indigestibility (%) | Digestibility (%) | Dry matter intake (g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup> ) |
|-----------|------------|----------------------------|-----------------------------|---------------------|-------------------|--|
| Apollo    |            |                            |                             |                     |                   |  |
|           | 1          | 8.1                        | 57.7                        | 14.0                | 86                | 40.02  |
|           | 2          | 7.6                        | 45.5                        | 16.7                | 83                | 43.51  |
|           | 3          | 8.6                        | 43.1                        | 20.0                | 80                | 28.09  |
|           | 4          | 10.1                       | 29.9                        | 33.8                | 66.2              | 21.15  |
|           | 5          | 7.6                        | 27.8                        | 27.3                | 72.7              | 28.04  |
|           | 6          | 7.7                        | 29.7                        | 25.9                | 74.1              | 28.12  |
| Mean ± SD |            | 8.3 ± 1.0                  | 39.0 ± 11.9                 | 23.0 ± 7.4          | 77.0 ± 7.4        | 31.50 ± 8.48   |
| Uno       |            |                            |                             |                     |                   |  |
|           | 1          | 10.4                       | 26.3                        | 39.5                | 60.5              | 21.55  |
|           | 2          | 7.5                        | 17.3                        | 43.4                | 56.6              | 19.61  |
|           | 3          | 6.5                        | 20.6                        | 33.6                | 68.4              | 26.97  |
|           | 4          | 9.0                        | 16.1                        | 55.9                | 44.1              | 15.17  |
|           | 5          | 7.1                        | 20.0                        | 35.5                | 64.5              | 21.77  |
|           | 6          | 9.0                        | 20.2                        | 44.6                | 55.4              | 17.49  |
| Mean ± SD |            | 8.3 ± 1.5                  | 20.1 ± 3.5                  | 41.7 ± 8.5          | 58.3 ± 8.5        | 20.42 ± 4.07   |
| Vegas     |            |                            |                             |                     |                   |  |
|           | 1          | 10.4                       | 37.6                        | 27.7                | 72.3              | 25.16  |
|           | 2          | 7.5                        | 39.0                        | 19.2                | 80.8              | 40.49  |
|           | 3          | 6.5                        | 31.7                        | 20.5                | 79.5              | 35.98  |
|           | 4          | 9.0                        | 34.5                        | 26.1                | 73.9              | 36.76  |
|           | 5          | 7.1                        | 27.6                        | 25.7                | 74.3              | 35.84  |
|           | 6          | 9.0                        | 18.4                        | 48.9                | 51.1              | 17.67  |
| Mean ± SD |            | 8.3 ± 1.5                  | 31.5 ± 7.6                  | 28.0 ± 10.8         | 72.0 ± 10.8       | 31.98 ± 8.69   |

Table 15. Mean fecal output and the associated 95% confidence interval

| Horse | Fecal output<br>(g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup> ) | 95% Confidence Limits |       |
|-------|--|-----------------------|-------|
| 1     | 8.26   | 7.85                  | 8.66  |
| 2     | 8.26   | 7.16                  | 9.37  |
| 3     | 7.62   | 6.99                  | 8.26  |
| 4     | 8.88   | 8.28                  | 9.48  |
| 5     | 8.16   | 7.93                  | 8.49  |
| 6     | 7.43   | 6.84                  | 8.02  |
| 7     | 6.77   | 5.81                  | 7.72  |
| 8     | 12.14  | 11.12                 | 13.15 |

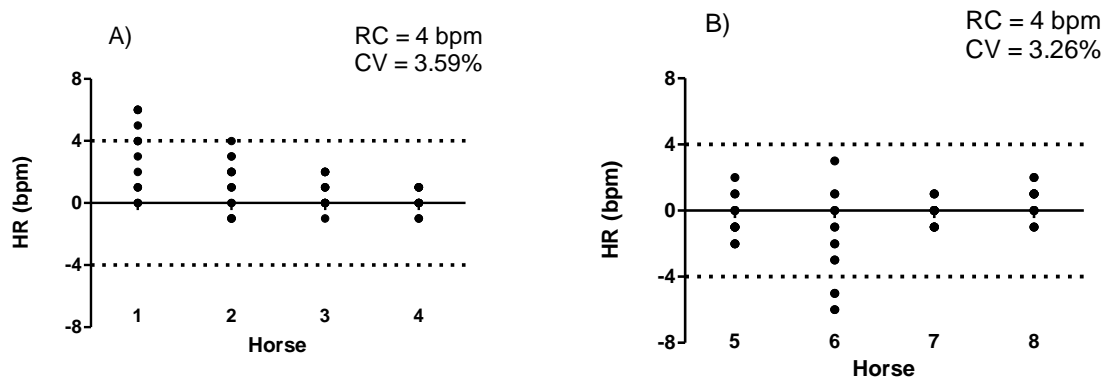
<sup>1</sup> Pooled fecal output from six daily measures

Table 16. Mean daily HR and the associated 95% confidence interval

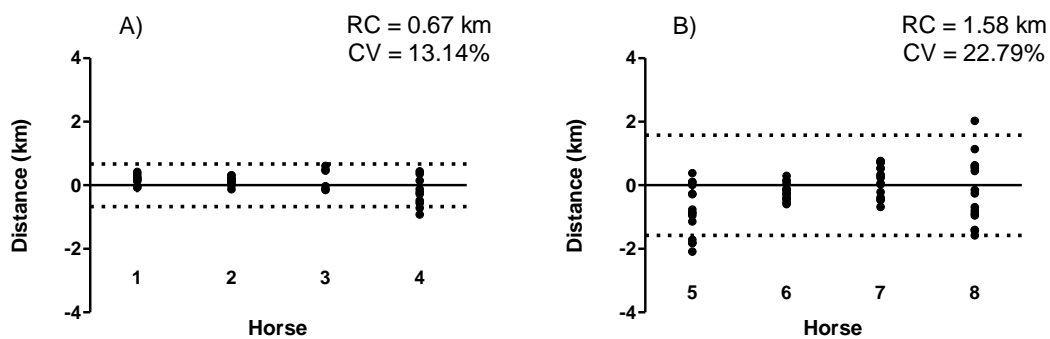
| Horse | HR<br>(bpm/d) | 95% CI |    |
|-------|---------------|--------|----|
| 1     | 43            | 40     | 46 |
| 2     | 45            | 43     | 46 |
| 3     | 41            | 40     | 41 |
| 4     | 42            | 42     | 43 |
| 5     | 40            | 39     | 41 |
| 6     | 39            | 36     | 41 |
| 7     | 42            | 42     | 43 |
| 8     | 41            | 40     | 42 |

Table 17. Mean daily distance traveled and the associated 95% confidence interval

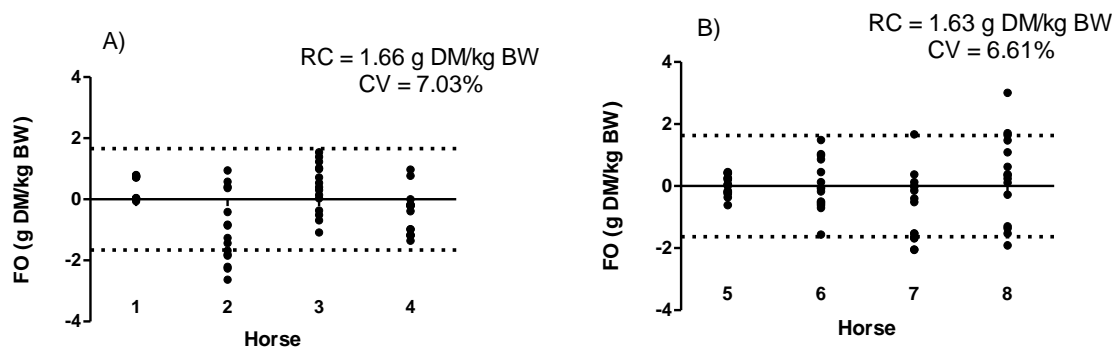
| Horse | Distance traveled<br>(km/d) | 95% CI |      |
|-------|-----------------------------|--------|------|
| 1     | 1.90                        | 1.68   | 2.11 |
| 2     | 2.06                        | 1.89   | 2.23 |
| 3     | 1.40                        | 0.93   | 1.86 |
| 4     | 1.84                        | 1.41   | 2.26 |
| 5     | 3.15                        | 2.33   | 3.98 |
| 6     | 2.27                        | 2.03   | 2.52 |
| 7     | 2.39                        | 2.05   | 2.74 |
| 8     | 2.23                        | 1.47   | 3.00 |



**Figure 6.** Comparison of fecal output between A) Group 1 and B) Group 2 within individual horse measurements over a 6-d collection period (n=15). The dotted horizontal line represents the repeatability coefficients of 1.66 g DM/kg BW and 1.63 g DM/kg BW, respectively, which are the maximum differences expected between two samples from the same horse 95% of the time.



**Figure 7.** Comparison of HR between A) Group 1 and B) Group 2 within individual horse measurements over a 6-d collection period (n=15). The dotted horizontal lines represent the repeatability coefficients 4 bpm, which is the maximum differences expected between two samples from the same horse 95% of the time.



**Figure 8.** Comparison of daily distance traveled between A) Group 1 and B) Group 2 within individual horse measurements over a 6-d collection period (n= 15). The dotted horizontal line represents the repeatability coefficients of 0.67 km and 1.58 km, respectively, which are the maximum differences expected between two samples from the same horse 95% of the time.

#### Literature Cited

- Sales, J. and G. P. J. Janssens. 2003. Acid-insoluble ash as a marker in digestibility studies: A review. *J. Anim. Feed Sci.* 12: 383-401
- Cuddeford, D. and D. Hughes. 1990. A comparison between chromium-mordanted hay and acid-insoluble ash to determine apparent digestibility of a chaffed, molassed hay/straw mixture. *Equine Vet. J.* 22: 122-125.
- Sunvold, G. D. and R. C. Cochran. 1991. Technical note: Evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash, and indigestible acid detergent fiber as internal markers for prediction of alfalfa, bromegrass, and prairie hay digestibility by beef steer. *J. Anim. Sci.* 69: 4951-4955.
- Thonney, M. L., B. A. Palhof, M. R. DeCarki, D. A. Ross, N. L. Firth, R. L. Quass, D. J. Perosio, D. J. Duhaime, S. R. Rolins, A. Y. M. Nour. 1985. Sources of variation of dry matter digestibility measurement by the acid insoluble ash marker. *J. Dairy Sci.* 68: 661-668.
- Orton, R. K., I. D. Hume, R. A. Leng. 1985. Effects of exercise and level of dietary protein on digestive function in horses. *Equine Vet. J.* 17: 386-390.
- Chavez, S. J., P. D. Siciliano, and G. B. Huntington. 2014. Intake estimation of horses grazing tall fescue or fed tall fescue hay. *J. Anim. Sci.* 92(5): 2304-2308.
- Olsson, N., and A. Ruudvere. 1955. The nutrition of the horse. *Nutr. Abst. Rev. Series A: Human Exper.* 25(1).
- Fleurance, G., P. Duncan, and B. Mallevaud. 2001. Daily intake and the selection of feeding sites by horses in heterogeneous wet grasslands. *Anim. Res.* 50(2): 149-156.

Cuddeford, D., and D. Hughes. 1990. A comparison between chromium-mordanted hay and acid-insoluble ash to determine apparent digestibility of a chaffed, molassed hay/straw mixture. *Equine Vet. J.* 22(2): 122-125.

Sutton, E. I., J. P. Bowland, and J. F. McCarthy. 1977. Studies with horses comparing 4N-HCl insoluble ash as an index material with total fecal collection in the determination of apparent digestibilities. *Can. J. Anim. Sci.* 57(3): 543-549.

Bergero, D., Miraglia, N., Abba, C., and M. Polidori. 2004. Apparent digestibility of Mediterranean forages determined by total collection of faeces and acid-insoluble ash as internal marker. *Livest. Prod. Sci.* 85(2): 235-238.

Miraglia, N., D. Bergero, B. Bassano, M. Tarantola, and G. Ladetto. 1999. Studies of apparent digestibility in horses and the use of internal markers. *Livest. Prod. Sci.* 60(1): 21-25.

Bergero, D., C. Préfontaine, N. Miraglia, and P. G. Peiretti. 2009. A comparison between the 2N and 4N HCl acid-insoluble ash methods for digestibility trials in horses. *Animal* 3(12): 1728-1732.

Peiretti, P. G., G. Meineri, N. Miraglia, M. Mucciarelli, and D. Bergero. 2006. Intake and apparent digestibility of hay or hay plus concentrate diets determined in horses by the total collection of feces and n-alkanes as internal markers. *Livest. Sci.* 100(2): 189-194.

Holland, J. L., D. S. Kronfeld, D. Sklan, and P. A. Harris. 1998. Calculation of fecal kinetics in horses fed hay or hay and concentrate. *J. Anim. Sci.* 76(7): 1937-1944.

Menard, C., P. Duncan, G. Fleurance, J. Y. Georges, and M. Lila. 2002. Comparative foraging and nutrition of horses and cattle in European wetlands. *J. Appl. Ecol.* 9(1): 120-133.

Dowler, L. E., P. D. Siciliano, S. E. Pratt-Phillips, and M. Poore. 2012. Determination of pasture dry matter intake rates in different seasons and their application in grazing management. *J. Equine Vet. Sci.* 32(2): 85-92.

Stevens, D. M., J. B. J. Van Ryssen, and J. P. Marais. 2002. The use of n-alkane markers to estimate the intake and apparent digestibility of ryegrass and Kikuyu by horses. *S. African J. Anim. Sci.* 2(1): 50-56.

Booth, M. E. 1998. Factors influencing the energy requirements in native ponies living outdoors in the United Kingdom. PhD Thesis. University of Edinburgh. 233.

Shingu Y. M. Kawai, H. Inaba, S. Kondo, H. Hata, and M. Okubo. 2000. Voluntary intake and behavior of Hokkaido native horses and light half-bred horses in woodland pasture. *J. Equine Sci.* 11(3), 69-73.

Eaton, M. D., D. L. Evans, D. R. Hodgson, and R. J. Rose. 1995. Effect of treadmill incline and speed on metabolic rate during exercise in thoroughbred horses. *J. Appl. Physiol.* 79(3): 951-957.

Coenen, M. 2005. About the predictability of oxygen consumption and energy expenditure in the exercising horse. Proc. 19th Meeting. Equine Science Society. Tuscon, AZ.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15(4): 371-372.

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44(2): 282-287.

Dulphy, J. P., W. Martin-Rosset, H. Dubroeuq, J. M. Ballet, A. Detour, and M. Jailler. 1997. Compared feeding patterns in ad libitum intake of dry forages by horses and sheep. *Livest. Prod. Sci.* 52(1): 49-56.

Marlow, C. H., E. M. Van Tonder, F. C. Hayward, S. S. Van der Merwe, and L. E. Price. 1983. A report on the consumption, composition and nutritional adequacy of a mixture of lush green perennial ryegrass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*) fed ad libitum to Thoroughbred mares. *J. S. African Vet. Assoc.* 54(3): 155-157.

Gill, J. C., S. E. Pratt-Phillips, R. A. Mansmann, and P. D. Siciliano. 2013. Estimated digestible energy intakes in moderate and overweight horses. *J. Equine Vet. Sci.* 33(5): 340.

Gehrke, E. K., A. Baldwin, and P. M. Schiltz. 2011. Heart rate variability in horses engaged in equine-assisted activities. *J. Equine Vet. Sci.* 31(2): 78-84.

Quinn, R. W., A. O. Burk, J. K. Suagee, and T. G. Hartsock. 2006. Equine obesity: implications for health and strategies for control. *Proceedings of the 4th Mid-Atlantic Nutrition Conference.* 23-36.

Cymbaluk, N. F., and G. I. Christison. 1990. Environmental effects on thermoregulation and nutrition of horses. *The Veterinary Clinics of North America Equine Practice* 6(2): 355-372.

Webb, S.P., G.D. Potter, J.W. Evans, and G.W. Webb. 1989. Influence of body fat content on digestible energy requirements of exercising horses in temperate and hot environments. *Proc. 11th Eq. Nutr. Physiol. Symp.* Stillwater, OK. 279-284.

National Research Council. 2007. *Nutrient Requirements of Horses.* 6<sup>th</sup> Revised Edition, National Academic Press. Washington, DC.

Geor, R. J., L. J. McCutcheon, G. L. Ecker, and M. I. Lindinger. 1995. Thermal and cardiorespiratory responses of horses to submaximal exercise under hot and humid conditions. *Equine Vet. J.* 27(S20): 125-132.

Duncan, P. 1985. Time-budgets of Camargue horses III. Environmental influences. *Behaviour* 92(1): 188-208.

Salter, R. E., and R. J. Hudson. 1979. Feeding ecology of feral horses in western Alberta. *J. Range Manag.* 221-225.

Hampson, B. A., J. M. Morton, P. C. Mills, M. G. Trotter, D. W. Lamb, and C. C. Pollitt. 2010. Monitoring distances travelled by horses using GPS tracking collars. *Austra. Vet. J.* 88(5): 176-181.

Walker, G. A. 2002. Common Statistical Methods for Clinical Research. 2<sup>nd</sup> vol. SAS Institute. Cary, NC.

Motulsky, H. J. 2007. Prism 5 Statistics Guide. GraphPad Software Inc. San Diego, CA.

Hoyt, R. W., T. E. Jones, T. P. Stein, G. W. McAninch, H. R. Lieberman, E. W. Askew, and A. Cymerman. 1991. Doubly labeled water measurement of human energy expenditure during strenuous exercise. *J. Appl. Physiol.* 71(1), 16-22.

Lawrence, L. 2009. Assessing energy balance. *Advances in Equine Nutrition IV*, 43.

Hodgson, D. R., K. H. McKeever, and C. M. McGowan. 2013. *The Athletic Horse: Principles and Practice of Equine Sports Medicine*. Elsevier Health Sciences.

Bland, J. M., and D. G. Altman. 1996. Statistics notes: measurement error. *BMJ* 313.7059: 744.

Ellard, M. E., and S. L. Crowell-Davis. 1989. Evaluating equine dominance in draft mares. *J. Appl. Anim. Behav.* 24(1): 55-75.

Boyd, L. E. 1991. The behavior of Prezewalski's horses and its importance to their management. *Appl. Anim. Behav. Sci.* 29(1-4): 301-318.

Pratt, S. E., P. D. Siciliano, and L. Walston. 2009. Variation of insulin sensitivity estimates in horses. *J. Equine Vet. Sci.* 29(6): 507-512.

## CHAPTER V. EXPERIMENT 4

### **The effect of time- and space-restricted grazing on body weight, body condition score, resting insulin concentration, and activity in grazing horses**

#### 5.1 Abstract

Excessive calorie intake from pasture contributes to obesity and increases the risk for laminitis. The objectives of this study were to determine if body weight (BW), body condition score (BCS), and insulin (INS) concentration could be significantly reduced if the time and space allotment for grazing was limited, and to determine the effect of limited pasture access on voluntary activity in horses. Ten mature, idle geldings were randomly assigned to either a time- and space-restricted grazing group (RG; BW  $584 \pm 70$  kg, Age 5 to 12 yrs, n=5) or a 24-h grazing group (CTRL; BW  $571 \pm 51$  kg, Age 5 to 12 yrs, n=5) for 35 d. The RG horses had access to pasture for 8 h/d and the grazing cell contained herbage availability to provide 80% of the mean NRC (2007) maintenance digestible energy (DE) requirement for a 7-d grazing period. The remaining 15 h/d was spent in individual dry lot pens with feed withheld. Horses in the RG group were moved to a new grazing cell every 7 d for 5 weeks. At the end of each week, all of the horses were brought inside overnight and at 7:00 AM the next day (d 7), scale BWs and BCSs were recorded and venous blood samples were collected for resting INS analysis prior to turnout. Horses alternated through two, 3-d collection periods of heart rate (HR) monitoring/distance traveled (DT) assessments by Polar V800 activity monitors. Weekly repeated measurements of BW, BCS, INS, and activity monitoring were analyzed using repeated measures ANOVA (PROC MIXED; TYPE=AR(1) and controlling for the within-horse variance) in SAS. Correlation between daily HR and DT was evaluated by Pearson's correlation coefficients. The weekly

herbage mass (HM) allowance and HM removal rate for RG over 5 weeks was 11, 12, 10, 17, 14, and 32 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup> and 71%, 40%, 71%, 59%, 66%, and 48%, respectively. Restricted grazing orses lost ( $p<0.001$ ) more BW and BCS over time than the CTRL horses. Weekly INS was unaffected by the treatment group ( $p=0.52$ ) or the treatment by time interaction ( $p<0.71$ ), but was significantly affected by time ( $p<0.012$ ). There was a positive correlation ( $r = 0.52$ ) between HR and DT when all horses were considered; RG reduced overall activity by approximately 30%, but did not ( $p<0.05$ ) reduce daytime grazing activity. Time and space restricted grazing reduced BW and BCS of horses while maximizing the utilization of available forage by promoting uniform grazing.

## 5.2. Introduction

Grazing forage is an important part of horse nutrition but energy intake from pasture can exceed the energy requirements of certain feeding classes of horses, leading to obesity. Weight reduction resulting from feeding hay-based diets providing 65% to 80% of maintenance energy requirements will result in weight reduction and an improvement in insulin sensitivity in the absence of pasture (Argo et al. 2012, McGowan et al. 2013, Ungru et al. 2012, Van Weyenburg et al. 2008). Some husbandry situations do not support complete or partial removal of obese horse from pasture for weight loss. Consequently, horse owners are limited to the use of grazing muzzles or time-restricted grazing.

Grazing muzzles present practical obstacles for horse owners, including opposition to use, trouble maintaining proper fit, or trouble catching the horse to apply the grazing muzzle, and their ability to efficiently restrict intake may depend on grass

species and muzzle design (Gill et al. 2016; Glunk et al. 2013, 2014). Although reducing time at pasture by  $\leq 9$  hr has resulted in reduced pasture dry matter intake (DMI) (Dowler et al. 2011, Glunk et al. 2013, Kennedy et al. 2008), animals are known to compensate by increasing grazing time (Allden and Wittaker 1970, Chacon and Stobbs 1976), intake rate (Dowler et al. 2011, Glunk et al. 2013, Kennedy et al. 2008), and bite rate (Kennedy et al. 2008); therefore, horses may consume similar intakes to 24-h grazing horses when  $> 9$  hr of pasture access is offered (if pasture supply is not limiting) (Siciliano 2012). Pasture herbage availability (HA) can impose a restraint on DMI in grazing animals. Allden and Whittaker (1970) found that when the HA diminished from 3000 kg/ha to 500 kg/ha, there was a fourfold reduction in the rate of consumption and a twofold increase in time spend grazing. At some HAs the increase in time spent grazing cannot compensate and therefore the total DMI is reduced. Spatial constraints impose an absolute restriction on DMI by reducing total herbage mass; however, there is limited data pertaining to the use of pasture size to restrict DMI in horses. It is suggested that managing the size of the paddock, stocking rate, and herbage availability are important components for proper ecological pasture management and grazing efficiency (Bott et al. 2013). Barnes et al. (2008) found that the heterogeneity of pasture utilization among intensive rotational grazing systems with different stocking rates was strongly positively associated with paddock size. Moate et al. (1999) found that as the herbage allowance increased, DMI increased curvilinearly in dairy cows; conversely, limiting herbage allowance through paddock size should result in decreased DMI in obese horses.

The objective was to determine if horses would decrease in BW, BCS, and basal INS concentration if the pasture herbage allowance was restricted to 80% of the maintenance DE requirements.

### 5.3 Materials and Methods

#### *5.3 a. Animals and Treatments*

The following experimental protocol was approved by the North Carolina State University Institutional Animal Care and Use committee. The experiment was conducted from October 12, 2015 to November 16, 2015 at the North Carolina State University Equine Research Unit in Raleigh, North Carolina.

Ten mature, idle, Quarter Horse geldings were randomly assigned to one of two treatment groups over a 35-d period: restricted grazing (RG; mean  $\pm$  standard deviation; BW:  $584 \pm 70$  kg; Age: 5 to 12 yr; n=5) and continuous grazing (CTRL; mean  $\pm$  standard deviation; BW:  $571 \pm 51$  kg; Age: 5 to 12 years; n=5). The RG group had access to a pasture area that was restricted in pasture area and time at pasture. Restricted grazing horses had access to pasture for 8 h/d beginning at 0800 and ending at 1600, and spent the remaining 15 h/d in individual dry lot pens (3.6 m x 12 m) with individual automatic watering troughs and no supplemental feed. Pasture area was determined as follows: the digestible energy restriction level was calculated as 80% of the (NRC) maintenance digestible energy requirement (DE; maintenance DE =  $33.3 \text{ kcal} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ ) based on the average BW for all five horses for a 7-d period. This restriction level (Mcal/d) was divided by the caloric content of the pasture (Mcal/ kg

DM) to equal the total forage requirement (kg DM) for the 7 days to meet the 80% restriction level.

The pasture herbage mass of the area was estimated using a falling plate meter, according to Rayburn and Rayburn (1998). The plate meter consisted of a circular plexi-glass disc, 0.25 m in diameter, attached to a polyvinyl chloride sheath covering labeled in 0.64 cm increments from 0 to 51 cm. For each measurement, the sheath was held up to the height of 51 cm and then dropped to the canopy below and the compressed canopy height was recorded. Twenty-five falling plate-meter measurements were taken from each of the RG cells in a serpentine pattern, with approximately 5 steps between each measurement so that the collection was random and the area was evenly represented. Initial plate meter measurements were taken the day prior to grazing a new pasture area to determine the mean initial compressed canopy height ( $x$ ), which was used to determine the diameter of the new, 7-d grazing cell.

Over the duration of study, 26 additional plate meter measurements, representing 9 short, 9 medium, and 8 tall compressed canopy heights, were collected from the entire pasture area to calibrate the plate meter. Calibration was accomplished by recording the compressed canopy height (cm) and then harvesting the plants within the compressed canopy's boundaries to 5 cm in height. The forage was harvested within a 0.25 m<sup>2</sup> polyvinyl chloride square frame using electric grass clippers (7.2 V cordless grass shear, Black + Decker, Baltimore, MD, USA). The harvested calibration samples were placed in separate cotton muslin bags, weighed, and oven-dried at 60 °C for a minimum of 48 h (until no weight change was detected). Grams of DM harvested within the 0.25 m<sup>2</sup> area under the plate meter were regressed against the compressed

canopy height (cm) to develop a regression equation to determine the pasture herbage density (Fig. 11). The pasture herbage density was multiplied by four to estimate the grams of DM per m<sup>2</sup> and then multiplied by 75% grazing efficiency to estimate the total herbage available for grazing in a meter square area. An initial herbage estimate was calculated using a grazing stick in order begin immediately after the measures were taken. The dimensions of each grazing cell (m<sup>2</sup>) were calculated the morning prior to grazing as follows:

$$\text{Cell dimensions (m}^2\text{)} = \frac{\text{Forage requirement (kg DM)}}{\text{Herbage density (g DM/m}^2\text{)}}$$

Grazing cells containing the 7-d pasture allocations were created by moving temporary electric fencing (1.27-cm poly tape). Horses were moved into a new cell every 7 d for 5 weeks. All horses had continuous access to water and salt.

Initial herbage mass (iHM) measurements were taken the day prior to grazing a new grazing cell and the residual herbage mass (rHM) measurements were taken the 7<sup>th</sup> day (last day of grazing) immediately after the horses were removed from pasture. Initial herbage mass (iHM) and rHM measurements were repeated over 5 wk. The removal rate within each grazing cell was estimated  $1 - (\text{rHM}/\text{iHM}) \times 100$ .

The CTRL horses continuously occupied a 155 m × 149 m area that was within and adjacent to the pasture containing grazing cells used for RG. The herbage mass available for grazing was estimated weekly by falling plate meter measurement and the iHM exceeded the amount necessary to meet maintenance DE requirements for the 5 horses over a 35-d period (38 g DM·kg BW<sup>-1</sup>·d<sup>-1</sup>).

### *5.3 b. Pasture Sample Collection, Dry Matter Determination and Pasture Analysis*

Plant composition of both pasture areas was estimated using the point-step method, according to Glunk et al. (2013), using 100 measurements. The percentage of each plant type and bare ground was calculated.

Pasture grab-samples were collected from CTRL and RG (ungrazed) on d 4, d 9, d 18 and d 25 at 1300 to determine the pasture DE concentration. The DE content was initially estimated using 2.25 Mcal DE/kg DM because there was a delay in the pasture analysis results from the outside lab. The pasture DE value was generated from previous analysis of the same pasture during the same season (Dowler et al. 2011; Glunk et al. 2013). Pasture samples were analyzed for chemical composition (DM, CP, ADF, NDF, fat, NFC, Ca, P, Mg, Na, Cu, Zn, Mn, Fe, DE, ash) by the North Carolina Department of Agriculture Forage Testing Laboratory & Consumer Services (Raleigh, North Carolina). Non-fiber carbohydrate (NFC) was calculated as %DM - %CP - %NDF - %Ash - % Fat. Pasture DE was calculated using Pagan's equation (1998):  $DE \text{ (kcal/kg DM)} = 2,118 + 12.18 (\%CP) - 9.37 \times (\%ADF) - 3.83 \times (\%hemicellulose) + 47.18 \times (\%fat) + 20.35 \times (\%NSC) - 26.30 (\%ash)$ , where hemicellulose = ADF - NDF, and non-structural carbohydrate (NSC) =  $(100 - \%NDF - \%Fat - \%Ash - \%CP)$ .

### *5.3 c. Animal Sample Collection and Analysis*

Scale body weights (BW) and body condition scores (BCS) were recorded immediately prior to the start of the study and at 7, 14, 21, 28, and 35 d at 7:00 am. Body weight was measured using an electronic large animal scale with  $\pm 1.0$  kg sensitivity (Smart Scale 200, Gallagher Animal Management, North Kansas, MO, USA). Body condition scores were determined by one observer according to the 1-9 point

scale using 1/2 units (Henneke et al. 1983). On the evening of the seventh day of each period, all of the horses were housed overnight in individual drylot pens. At 7:00 am on 0, 7, 14, 21, 28, and 35 d, a jugular venous blood sample was collected from the ten horses prior to 8:00 am turnout. The harvested serum and plasma blood samples were stored at -20 °C until further analysis. Serum insulin was analyzed in duplicate assays using a commercially available Insulin radioimmunoassay kit (ImmuChem Insulin<sup>125</sup> RAI, MP Biomedicals, LLC., Orangeburg, NY, USA). Repeated assays were run for the samples. The lowest detectable limit of the assay was 5.5 µU/mL. The intra-assay coefficient of variation was 7%.

#### *5.3 d. Distance Traveled and Heart Rate Monitoring*

Polar V800 (Polar Electro Inc., Lake Success, NY, USA) equine activity monitors were used to measure daily distance traveled (DT) and average heart rate (HR). Because there was only 5 activity monitors, horses from the RG and CTRL groups were randomly assigned to two group (Group 1 (RG = 3, CTRL = 2) and Group 2 (RG = 2; CTRL = 3) and rotated through two, 72 h periods that were spaced two weeks apart (Table 18). Period 1 of activity monitoring occurred over the second week of the trial (d 7 to d 12) and the second period (Period 2) was completed 2 weeks thereafter (d 21 to d 26). The Polar Equine V800 monitors were attached by the horses as follows: the HR electrode band was secured around the girth area of the horse and pinned in place; the receiver component was secured with vet wrap to the top of the belt (next to the withers on the left side), synced, and started. Water and ultrasound gel were continuously applied to the electrode region to maintain conductivity and the girth area of the horses was clipped using eclectic horse clippers to increase skin contact. All

receivers were started at 8:00 am and removed at 4:00 pm. Receivers from the CTRL horses were charged and put back on the horses for the nighttime (12 h) recording, and the nighttime (12 h) recording for the RG horses was collected once while in the dry lot pens to represent any stall-related activity. The daytime (8:00 am to 4:00 pm) and nighttime (6:00 pm to 6:00 am) activity data was logged and downloaded to the software program (PolarFlow.com). Data collected from the receivers included: date, time, day of study, period of feed (daytime or nighttime), device feed (normal, low battery, paused, or not running), position of device (normal or shifted), start-time, end-time, duration of feed (hr), distance traveled (km), average speed (km/h), average heart rate (bpm), temperature, weather, and mood of horse. The Polar V800 error rate for distance was 0.26% and 1.96% for trueness and accuracy, respectively (fellrnr.com).

### *5.3 e. Statistical Analysis*

Weekly repeated measurements of BW, BCS, and resting insulin (INS) concentration were analyzed using analysis of variance (ANOVA) for repeated-measures design in PROC MIXED using a first-order autoregressive (TYPE=AR(1)) and variance component covariate structures (a random horse statement) in SAS (Walker, 2002). The most appropriate covariate structure was determined by selecting the structure with the model that yielded the lowest Akaike information criterion, Schwarz Bayesian criterion, and -2 res log likelihood values (Walker 2002; Walker and Shostak 2010). The model statement included the effects of treatment, time, and the treatment by time interaction, with the repeated measure of time and horse as the subject. One-way ANOVA for completely randomized designs using the PROC MIXED procedure in SAS was selected for the dependant variables of HR and DT and contained the model

parameters horse and period (period 1 = initial 3 d of collection; period 2 = final 3 d of collection) within a treatment (RG and CTRL). A separate model testing the effect of Group was not significant and therefore not included in the model. The correlation between HR and DT was evaluated according to Pearson's correlation coefficient using GraphPad Prism software (Motulsky 2007). A p value of <0.05 was considered significant.

## 5.4 Results

### 5.4 a. Pasture Composition

The pasture composition consisted of 60% non-toxic endophyte-infected tall fescue (*Lolium arundinaceum* Schreb cv Max-Q, Pennington Seed, Madison, GA), 25% crabgrass (*Digitaria ischaemum*), 6% annual bluegrass (*Poa annua*), 6% bare ground, with the remaining 1% of each of the following: fox tail (*Alopecurus myosuroides*), Carolina horsenettle (*Solanum carolinense*), and clover (*Trifolium*). The chemical composition of the pasture is shown in Table 19. There was no significant ( $p>0.05$ ) difference in DE content between treatments.

The herbage mass calibration equation yielded a final prediction equation of  $11.05x$  ( $p<0.001$ ;  $n=26$ ) when the intercept was forced through zero. The model well described the relationship between pasture compressed canopy height and herbage mass ( $n = 26$ ;  $p<0.001$ ;  $R^2=0.66$ ; Fig. 14). Mean initial herbage mass (kg/d and g DM·kgBW<sup>-1</sup>·d<sup>-1</sup>), residual herbage mass (kg DM), herbage mass removal (kg DM), and estimated DE intake (Mcal/d and % DE requirement) for RG are shown in Table 20. Restricted grazing cells 4 and 5 were made larger than the targeted DE allowance to

account for excess trampling because of wet and muddy conditions. Horses were consequently moved to cell 6 one day prior to the end of the study because of overgrazing of cell 5. The dimensions of cell 6 were made large enough to accommodate all 5 horses for one day. The weekly HM removal rate for RG was 71%, 40%, 71%, 59%, 66%, and 48%, respectively. The mean weekly herbage mass (kg DM), herbage mass removal (kg DM), removal rate (%) and estimated DE intake (Mcal/d and % DE requirement) for CTRL are shown in Table 21. The iHM of the CTRL pasture on d 0 supplied  $38 \text{ g DM} \cdot \text{kgBW}^{-1} \cdot \text{d}^{-1}$ , which was in excess of the mean DE requirement (NRC 2007; Table 22). Overall, CTRL horses consumed more forage than RG and the difference in estimated DE intake (calculated from the herbage removal rate) was greater ( $p < 0.001$ ) for CTRL than RG.

#### *5.4 b. Change in Body Weight, Body Condition, and Insulin Concentration*

There was no difference in initial BW or BCS between treatment groups ( $p = 0.63$ ; 12 kg). Mean BW was affected by time ( $p < 0.001$ ) and the treatment by time interaction ( $p < 0.001$ ), but not affected by treatment ( $p = 0.68$ ; Fig. 9). The CTRL increased ( $p = 0.001$ ) in BW from d 0 to d 7 ( $7.4 \pm 2.3 \text{ kg}$ ) and d 7 to d 14 ( $10.0 \pm 2.3 \text{ kg}$ ) and then remained unchanged ( $p > 0.05$ ) from d 14 to d 21 ( $-3.2 \pm 2.3 \text{ kg}$ ), d 21 to d 28 ( $-0.4 \pm 2.3 \text{ kg}$ ) and d 28 to d 35 ( $0.8 \pm 2.3 \text{ kg}$ ). The RG decreased ( $p < 0.0001$ ) in BW from d 0 to d 7 ( $-16.8 \pm 2.3 \text{ kg}$ ), remained unchanged ( $p > 0.05$ ) from d 7 to d 14 ( $-0.6 \pm 2.3 \text{ kg}$ ) and d 14 to d 21 ( $-3.6 \pm 2.3 \text{ kg}$ ), and then decreased ( $p < 0.05$ ) again from d 21 to d 28 ( $-8.6 \pm 2.3 \text{ kg}$ ) and d 28 to d 35 ( $-5.4 \pm 2.3 \text{ kg}$ ). The differences in the means between RG and CTRL increased over time from d 7 (12 kg) to d 35 (37.4 kg). Mean BCS was affected by the treatment by time interaction ( $p < 0.01$ ), but was not affected by

treatment ( $p=0.27$ ) or time ( $p=0.21$ ; Fig. 9). The difference in the means between RG and CTRL increased over time from d 0 (0.1 BCS) to d 35 (2.0 BCS). Mean ( $\pm$ SD) BW of RG decreased in BW by  $-35 \pm 4$  kg, whereas CTRL increased in BW by  $14 \pm 6$  kg. Mean ( $\pm$ SD) BCS of RG declined by  $-1.0 \pm 0.5$ , whereas CTRL increased by  $0.5 \pm 1.0$ . There was no effect of treatment ( $p=0.524$ ) or the treatment by time interaction ( $p=0.710$ ) on INS; however there was an effect of time on INS ( $p=0.012$ ) (Fig. 10). The CTRL increased ( $p=0.049$ ) in INS concentration from d 0 to d 7 ( $8.5 \pm 4.2$   $\mu$ U/mL) and then remained unchanged ( $p>0.05$ ) from d 7 to d 14 ( $-8.1 \pm 4.2$   $\mu$ U/mL), d 14 to d 21 ( $3.0 \pm 4.2$   $\mu$ U/mL), d 21 to d28 ( $-3.2 \pm 4.2$   $\mu$ U/mL) and d 28 to d 35 ( $4.7 \pm 4.2$   $\mu$ U/mL). The RG remained unchanged ( $p>0.05$ ) in INS concentration from d 0 to d 7 ( $6.8 \pm 4.2$   $\mu$ U/mL), d 7 to d 14 ( $-4.4 \pm 4.2$   $\mu$ U/mL), d 14 to d 21 ( $-3.1 \pm 4.2$   $\mu$ U/mL), and d 21 to d 28 ( $-5.0 \pm 4.2$   $\mu$ U/mL) and then increased ( $p<0.01$ ) from d 28 to d 35 ( $12.3 \pm 4.2$   $\mu$ U/mL).

#### *5.4 c. Activity Monitoring*

Figure 11 shows the difference in activity between treatments for initial and final periods of activity monitoring. For period 1, mean ( $\pm$  SEM) HR was greater ( $p<0.001$ ) for CTRL ( $45 \pm 1$  bpm) than RG ( $37 \pm 1$  bpm) and in period 2, mean ( $\pm$ SEM) HR for CTRL decreased to  $41 \pm 1.0$  bpm, but remained greater ( $p<0.001$ ) than RG ( $37 \pm 1$ ). For period 1, mean ( $\pm$ SEM) for DT was greater ( $p<0.001$ ) for CTRL ( $1.94 \pm 0.17$  km) than RG ( $1.13 \pm 0.17$  km), and in period 2, mean ( $\pm$  SEM) DT for CTRL decreased to  $1.73 \pm 0.17$  km, but was still greater than RG ( $1.27 \pm 0.17$ ). CTRL horses decreased ( $p<0.001$ ) in HR and DT from period 1 to period 2, whereas RG remained constant in HR and increased ( $p<0.001$ ) in DT from period 1 to period 2. There was a significant ( $p<0.0001$ ) difference between horses in HR and DT (Table 22). Overall, RG reduced

activity by approximately 30%; however there was no difference ( $p=0.73$ ) in daytime grazing activity between RG and CTRL (Fig. 12). There was a positive correlation ( $r=0.52$ ;  $p<0.001$ ) between HR and DT when all horses were considered (Fig. 13).

## 5.5 Discussion

Results from this study showed that time-and space-restricted grazing can be used to restrict pasture intake in horses, which was demonstrated by a significant reduction in BW and BCS in the RG horses. On average, horses in the RG group lost approximately  $6.0 \pm 0.5\%$  of initial BW and decreased by 1 BCS, whereas CTRL horses gained approximately  $2.5 \pm 1.0\%$  of initial BW and increased by 0.5 BCS. These results support the development of a successful weight management program for pasture-fed horses.

The current results were similar to those of McGowan et al. (2013) during a six week study feeding 1.25% of BW in DMI of soaked foraged and a vitamin/mineral supplement (providing approximately 67% of maintenance DE requirements). Horses lost a mean of 6.8% in BW and BCS significant decreased by 0.5 units. The mean BW loss for RG the first week was  $17 \pm 2.1$  kg ( $2.9 \pm 0.3\%$  of initial BW) and averaged approximately 0.8% of BW thereafter. Other studies have found that the rate of weight loss is greatest during the first week of dietary restriction from changes in gut full and therefore water retention. Overnight fasting and changes in gut fill have been associated with a 5% decrease in BM in horses and ponies (Webb and Weaver 1979). Argo et al. (2012) and Dugdale et al. (2010) reported that animals undergoing calorie restriction lost 4.52% and 4.3% of BW, respectively. It is also possible that the depletion of

glycogen reserves occurred in response to the transition from *ad libitum* feeding to negative energy balance. After the first week, weekly BW declined by  $0.80 \pm 0.60\%$ , which was greater than mean weekly weight reduction that was reported in studies that fed a targeted intake level over the duration of study (Argo et al. 2012, Dugdale et al. 2010). One study reported the successful reduction of BW by feeding a decreasing level of intake, targeting a mean weekly rate loss of approximately 1% (Van Weyenburg et al. 2007). Therefore, the rate of weekly rate loss after the initial week is likely dependent on factors related to the restriction level.

An 80% restriction level of the National Research Council's maintenance DE requirement ( $33.3 \text{ kcal} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ ) was chosen for this study based on previous weight loss studies in stall-fed horses (Argo et al. 2012, Dugdale et al. 2010), a safe level of restriction to reduce any possible digestive complications, and a level of intake that would be able to be maintained for 7 days while not damaging the pasture. This level of restriction was able to be maintained from d 0 to d 20. From d 21 to d 29, periods of rain caused the pasture conditions to deteriorate and the grazing cells became muddy and heavily trampled. To reduce any permanent damage to the pastures and to accommodate the loss of pasture from trampling, the temporary fence was extended out mid-week or the horses were moved to a new grazing cell early. Thus, the HM allowance appeared greater from d-21 to d 35; however, the RG horses continued to decline in BW from d 21 to d 35, so it can be assumed that the actual DMI (and therefore digestible energy intake) was less than the predicted amount shown for cells 4 and 5. The herbage removal rate provides further evidence that the horses did not consume excess calorie intake over those days because the estimated mean digestible energy

intake amount was similar to the DE restriction level. On the last day of study, the horses were moved to cell 6 because there was not enough forage remaining in cell 5. The dimensions of cell 6 were based on the minimum space to accommodate the 5 horses without conflict for one day, which resulted in an estimated iHM and DE allowance that was above the restriction level. Based on the herbage mass removal rate, it appeared that the horses did not consume pasture in excess of requirements for the single day of grazing.

The time- and space-restricted grazing system maximized the utilization of available forage by promoting uniform grazing. Each week, the RG horses removed pasture completely and efficiently. Herbage mass removal was calculated by subtracting the residual herbage mass from the initial herbage mass. This method only estimates intake because it does not account for regrowth, trampling, and other factors that could potentially affect compressed canopy height. Most of the cells were grazed to a mean height of 3.0 cm to 6.5 cm. Because the HM calibration equation includes a minimum height of 4 cm, it is possible that pasture height estimated below this value would result in overestimated rHM; however, any deviation would be minimal based on the values measured in this study. Although the cells were grazed to a height of less than 6.5 cm, the vegetation was not damaged beyond the point of regrowth and manure was evenly scattered throughout the area (Bott et al. 2013). When the fence was moved out mid-week to accommodate the inclement weather, it was observed from the Polar V800 GPS recordings that the horses consumed pasture only in the new, ungrazed section. Because the old section of pasture was not grazed during those days (and the wet conditions favored re-growth), the height of the pasture from these cells (cell 4 and

cell 5) had the potential to be greater than the other cells that were evenly grazed for 7 d. The range in HM removal rate (%) was similar to the values reported in Chavez et al. (2013) (39% and 80%). The large range in removal rates and therefore potential intake shows that this method of estimating intake contains some error; however it is likely that horses consumed fresh forage at or below the 80% DE restriction level.

On the contrary, CTRL exhibited an overall herbage removal rate of 72% and DMI rate of  $21 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  when continually occupying a large pasture area supplying in excess of maintenance DE requirement. Overall, the mean estimated DE intake was significantly greater than that of the RG group. On average, CTRL exhibited DE intakes of approximately 150% above the mean maintenance DE requirement level. When considering the mean DE content of the control pasture ( $2.45 \text{ Mcal DE/kg DM}$ ), an average DMI rate of  $20 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  would equal approximately 150% of maintenance requirements, which matches the overall estimation of DE intake for the CTRL horses. This consumption of pasture in excess of maintenance requirements explains the increase in BW and BCS that was observed in the CTRL horses. The lowest week of HM removal occurred during a week of heavy rain, but the horses appeared to have increased their intake rate the following week to compensate.

The HM allowance of the RG group supported a similar intake level to 7 h to 8 h of grazing per day reported in previous study ( $1.3$  to  $1.5 \text{ g DM} \cdot \text{kg BW} \cdot \text{h}$ ;  $10.4$  to  $12 \text{ g DM} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ ) (Catillon 1986, Dowler et al. 2012). The initial HM allowance from d-0 to d-20 varied from 1.10% to 1.30% of BW in DM. Because the DMI rate is not linear across hours of restricted grazing, it is probable that DMI is not linear across days of restricted grazing. Collas et al. (2015) found that the DMI of grazing horses was

accelerated when the herbage mass availability increased from 35 to 70 g DM·kgBW<sup>-1</sup>·d<sup>-1</sup>.

1. Consequently, the intake rate of RG may have been accelerated at the beginning of the week when the sward height was tall (33 to 36 cm), leaving little to eat towards the end of the week when the sward height was considerably shorter (5 to 8 cm). The effect of horses rotating between lush and sparse pasture over a course of a month has not been evaluated for potential health risk; however the authors do caution that a possible disturbance in gastrointestinal microflora and insulin concentration could result from these abrupt changes in intake rate and forage composition, particularly pertaining to irregularity in non-structural carbohydrate load.

It is recommended that horses are offered a minimum of 1.0% of BW in roughage per day to maintain proper gut health and reduce the risk of colic or stomach ulcers (Geor 2010). Previous weight loss studies have emphasized that the minimum intake level should not drop below 1.0% of BW in DM. Diet restriction of this magnitude typically limits the expression of normal feeding activity and may result in the development of undesirable behaviors and/or GI complications. It was noted that some of the horse ate the wood paneling in the dry lot pens when feed was withheld for 15 h/d. Horses are known to increase wood-chewing during confinement (Krzak et al. 1991, Waters et al. 2002) or when limit-fed (Hothersal et al. 2009). Wood-chewing infrequently causes small intestinal obstruction because of the ingestion of wood splinters (Green and Tong 1988, NRC 2007). A study by Curtis et al. (2011) found that obese ponies consumed a significant (>1 kg/d) amount of wood shavings when undergoing dietary restriction of 1.25% of BW. The ingestion of large quantities of straw bedding in horses has been identified as a risk factor for impaction colic (Reeves

et al. 1996). Additional risk factors of feeding infrequent large meals include altered hindgut fluid balance because of the changes in osmolarity that result from accelerated fermentation, both potentially contributing to an increased risk of colic. However, Siciliano et al. (2012) found that restricted grazing horses exhibited similar hindgut pH and fluid balance as those grazing for 24 h/d. In the present study, all of the horses remained healthy throughout the trial, with the exception of a mild colic episode in one obese horse on d-34 in the RG group, with a history of occasional colic. For horses more prone to gastrointestinal complications, the authors suggest implementing a more moderate restriction level and possibly the inclusion of hay during confinement.

Both treatment groups represented an equally mixed sample of body conditions from moderate to obese. Despite differences in initial BCS within the treatment groups, horses exhibited similarity in BW and BCS changes within-each group. Initial body weight of the RG group ranged from 480 kg to 661 kg, with the fattest horses weighing the greatest amount. Because the restriction level was based on the mean BW of the group, the mean restricted calorie requirement may have been more restrictive for the fattest horses. However, it can be also assumed that the most obese horses (n=2) have a reduced calorie requirement since 15% to 25% of their BW was composed of relatively metabolically inert adipose tissue and then only 57-85% of actual BW required feeding (Dugdale et al. 2010). Some studies have noted a resistance to weight loss in obese ponies (Argo et al. 2012); however this was not the case for the present study. Perhaps a combination of the high level of restriction and the ability to engage in voluntary pasture activity assisted in weight loss.

There were no significant differences in resting INS between treatments, although INS differed over time. The pattern of insulin concentration greatly mirrored the non-fiber carbohydrate level of the pastures, which has been seen in previous study (Fig. 15). Horses exhibited INS concentrations with considerable week-week variation. Pratt-Phillips et al. (2009) reported considerable variability (CV>30%) in INS for individual horses over a two-week sampling period in non-fasted horses. Four of the five RG horses increased in INS from d 0 to d 35. Mean resting INS for each horse was greater than the 20 uU/mL indicator of INS resistance, regardless of treatment group or BCS, with the exception of horse 2 and horse 8 (Frank et al. 2006, Hoffman et al. 2003). Resting INS concentration was highest for the most obese horses (BCS 8/9); horse 3 and horse 5 exhibited mean INS of 41.19 uU/mL and 43.12 uU/mL, respectively. Weight reduction has achieved a significant decrease in basal INS concentration in horses and ponies with the exclusion of pasture (Argo et al. 2012, Dugdale et al. 2011, NRC 2007). However, Gentry et al. (2002) found that weekly INS was not affected by feed restriction in two groups of broodmares: those receiving diets that maintained the body condition and those fed restricted diets that decreased body condition from moderate-obese (6.5 to 8.0) to lean (3.0 to 3.5). Similar unresponsiveness has been noted in area-under-the-curve INS from 10-h versus 24-h grazing in horses (Pratt-Phillips et al. 2013).

It is noteworthy to point out that the mean INS of the RG group was statistically unchanged from d 0 to d 28 and then 4 of the 5 horses increased in INS from d 28 to d 35 to a level that was greater than d 0. Although unlikely to exhibit a profound effect on resting INS concentration, the horses were moved to cell 6 the day prior to blood collection. Normally, blood collection took place prior to turnout on a new cell, after

horses had been grazing very sparse pasture for 1 to 2 days. Since cell 6 provided lush pasture, containing dry matter in excess of requirements, it is possible that horses overate, causing glucose and subsequent INS concentration to increase exponentially. The day-day effect on insulin concentration from successively grazing sparse pasture and then transitioning to lush pasture (in excess of demand) has not been investigated and warrants further study.

Grazing presents an opportunity for horses to expend more energy than in a stall. Horses with 24-h pasture exhibited greater activity than the RG horses, reflected by higher HR (5 bpm) and DT (30%). Horses with continuous turnout have more opportunity for activity than those that are partially confined to a stall; however when daytime pasture-associated activity was accessed, there was no difference in DT between RG and CTRL. This indicates that RG horses traveled similar distances as CTRL to consume pasture yet occupied a considerably smaller area. The data confirms that horse will increase activity to consume their maintenance requirement even when space and HA is restricted. Furthermore, results from the ANOVA analysis showed that RG appeared to travel a similar distance to the CTRL overall, during the final period of collection. It is possible that CTRL decreased their activity during the final collection period because of the rain (Rogalski, 1975), while RG horses increased their activity because of hunger associated with the increased duration of the treatment. Allden and Wittaker (1970) have found that horses with a restricted herbage allowance compensated by increasing their rate of consumption and time spent grazing.

Some limitation on the accuracy of activity tracking existed because of the cooler temperatures and thicker coats. The main problem was maintaining the conductivity of

the electrode band because the horses were not sweating. Horse 3 exhibited the most problems related to electrode conductivity, and the HR readings were unusually low in comparison with the other horses. Clipping the girth area and applying salt water to the electrode area on the girth strap increased the conductivity for the other horses, with the exception of horse 3.

This study presents a novel method for managing the body weight of horses on pasture through the use of an 8-h time-restricted and space-restricted grazing system. Horse managers can partition areas of pasture for horses requiring weight management and should restrict time at pasture to 8 h or less. This system represents a management technique that can be carried out for the management of ‘easy-keeper’ horses on pasture.

Table 18. Experimental Design for Daily Heart Rate and Distance Traveled

|           |     | Group 1             |      |    |    |      | Group 2             |      |    |    |      |
|-----------|-----|---------------------|------|----|----|------|---------------------|------|----|----|------|
| Horse     |     | 2                   | 4    | 5  | 8  | 9    | 1                   | 3    | 6  | 7  | 10   |
| Treatment |     | RG                  | CTRL | RG | RG | CTRL | CTRL                | CTRL | RG | RG | CTRL |
|           | Day |                     |      |    |    |      |                     |      |    |    |      |
| Period 1  | 1   | Activity monitoring |      |    |    |      |                     |      |    |    |      |
|           | 2   | Activity monitoring |      |    |    |      |                     |      |    |    |      |
|           | 3   | Activity monitoring |      |    |    |      |                     |      |    |    |      |
|           | 4   |                     |      |    |    |      | Activity monitoring |      |    |    |      |
|           | 5   |                     |      |    |    |      | Activity monitoring |      |    |    |      |
|           | 6   |                     |      |    |    |      | Activity monitoring |      |    |    |      |
| Period 2  | 1   | Activity monitoring |      |    |    |      |                     |      |    |    |      |
|           | 2   | Activity monitoring |      |    |    |      |                     |      |    |    |      |
|           | 3   | Activity monitoring |      |    |    |      |                     |      |    |    |      |
|           | 4   |                     |      |    |    |      | Activity monitoring |      |    |    |      |
|           | 5   |                     |      |    |    |      | Activity monitoring |      |    |    |      |
|           | 6   |                     |      |    |    |      | Activity monitoring |      |    |    |      |

Table 19. Pasture Chemical Composition <sup>a</sup>

| Item   | Day   |       |       |       |       |       |       |       |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|
|        | 4     |       | 9     |       | 18    |       | 25    |       |
|        | RG    | CTRL  | RG    | CTRL  | RG    | CTRL  | RG    | CTRL  |
| DM     | 26.18 | 26.24 | 33.27 | 31.78 | 25.14 | 30.16 | 22.29 | 27.63 |
| CP,%   | 16.88 | 19.61 | 16.09 | 17.19 | 18.78 | 16.53 | 21.53 | 19.00 |
| NDF,%  | 53.86 | 49.61 | 43.32 | 45.11 | 41.16 | 51.53 | 44.29 | 54.09 |
| ADF,%  | 30.42 | 28.36 | 24.52 | 24.94 | 23.32 | 28.84 | 24.01 | 30.05 |
| DE     | 2.22  | 2.4   | 2.62  | 2.55  | 2.6   | 2.55  | 2.51  | 2.30  |
| NFC,%  | 19.17 | 20.01 | 30.64 | 27.42 | 28.66 | 21.92 | 23.23 | 17.24 |
| Fat,%  | 3.24  | 3.53  | 3.4   | 3.4   | 3.88  | 3.64  | 3.42  | 3.05  |
| Ca,%   | 0.38  | 0.36  | 0.39  | 0.35  | 0.38  | 0.33  | 0.37  | 0.31  |
| P,%    | 0.37  | 0.33  | 0.33  | 0.29  | 0.33  | 0.27  | 0.36  | 0.31  |
| Mg,%   | 0.31  | 0.32  | 0.29  | 0.3   | 0.31  | 0.27  | 0.3   | 0.39  |
| Na,%   | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| K,%    | 2.44  | 2.83  | 2.22  | 2.53  | 2.81  | 2.23  | 2.86  | 2.35  |
| Cu,ppm | 8     | 9     | 6     | 6     | 6     | 6     | 8     | 7     |
| Fe,ppm | 69    | 86    | 89    | 79    | 66    | 88    | 77    | 147   |
| Mn,ppm | 98    | 112   | 83    | 92    | 90    | 91    | 75    | 103   |
| Zn,ppm | 37    | 37    | 24    | 28    | 22    | 22    | 27    | 31    |
| Ash,%  | 6.86  | 7.24  | 6.55  | 6.88  | 7.52  | 6.39  | 7.53  | 6.62  |

<sup>a</sup>All values are expressed on a 100% DM basis. Each value represents a single analysis from grab samples taken from each respective pasture area on the designated day. DE = digestible energy (Mcal/kg), CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, NFC = non-fibrous carbohydrate. Digestible energy was calculated according to Pagan (1998). Non-fibrous carbohydrate was calculated as 100 – (CP + NDF + fat + ash). RG: Restricted grazing; CTRL: Continuous grazing

Table 20. Herbage allowance, Digestible Energy (DE) allowance, Initial Herbage Mass (iHM), Residual Herbage Mass (rHM), and Estimated DE Intake for the Restricted Grazing Group

|  | Day   | Herbage allowance <sup>a</sup><br>(g DM·kg BW <sup>-1</sup> ·d <sup>-1</sup> ) | DE allowance<br>(% DE req.) | iHM <sup>b</sup><br>(kg DM) | rHM<br>(kg DM) | HM Removal<br>(kg DM) | Estimated DE Intake <sup>c</sup><br>(Mcal/d) | Estimated DE Intake<br>(% DE req.) |
|--|-------|--|-----------------------------|-----------------------------|----------------|-----------------------|--|------------------------------------|
| Cell 1<br>(1774 m <sup>2</sup> )             | 0-6   | 11   | 80                          | 233                         | 67             | 166                   | 10.67  | 55                                 |
| Cell 2<br>(1609 m <sup>2</sup> )             | 7-13  | 12   | 80                          | 237                         | 141            | 93                    | 6.91   | 37                                 |
| Cell 3<br>(1359 m <sup>2</sup> )             | 14-20 | 10   | 80                          | 201                         | 57             | 144                   | 10.50  | 56                                 |
| Cell 4*<br>(1914 m <sup>2</sup> )            | 21-26 | 17   | 138                         | 299                         | 124            | 175                   | 15.16  | 81                                 |
| Cell 5*<br>(1854 m <sup>2</sup> )            | 27-34 | 14   | 102                         | 300                         | 96             | 204                   | 13.26  | 72                                 |
| Cell 6 <sup>‡</sup><br>(558 m <sup>2</sup> ) | 35    | 32   | 247                         | 90                          | 47             | 43                    | 8.60   | 53                                 |

<sup>a</sup>The herbage allowance was estimated by dividing the initial herbage mass (g DM) by 5 horses, 7 days, and mean BW (kg).

<sup>b</sup>Initial HM was calculated according the herbage mass prediction equation and a 75% grazing efficiency.

<sup>c</sup>DE intake was estimated by dividing the HM removed by the 5 horses and 7 day and multiplying by the pasture DE content.

\*Excessive trampling from wet/muddy conditions; the fence was moved out mid-week.

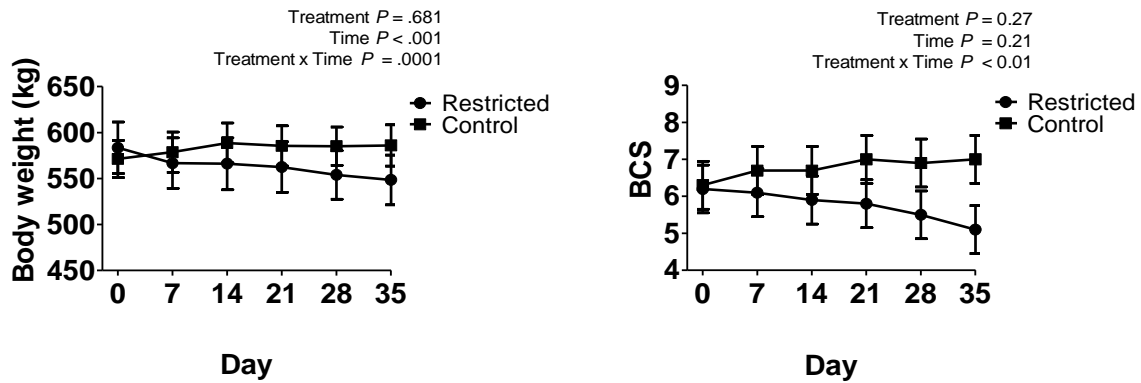
<sup>‡</sup>Horses were moved to a new cell on d 35 because of overgrazing.

Table 21. Herbage Mass (HM), HM Removal, and Estimated Digestible Energy (DE) Intake for the Control Group

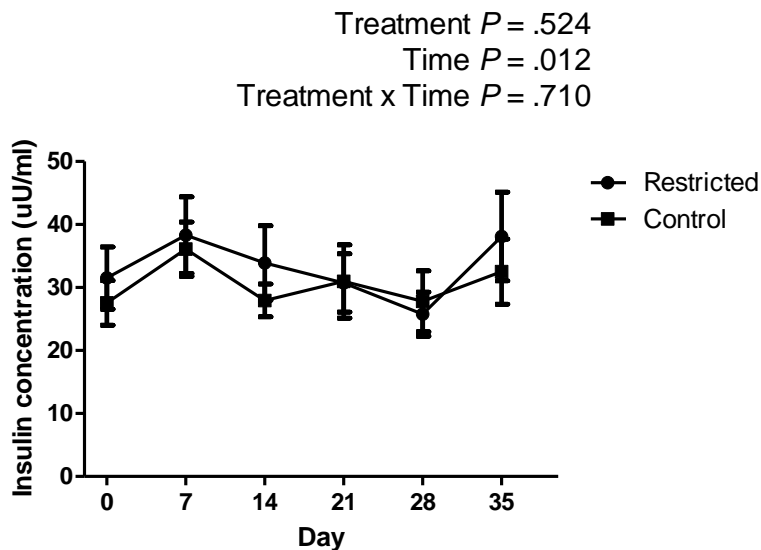
| (Day) | iHM<br>(kg DM) | rHM<br>(kg DM) | HM<br>Removal<br>(kg DM) | Estimated DE<br>Intake<br>(Mcal/d) | Estimated DE<br>intake<br>(% DE req.) |
|-------|----------------|----------------|--------------------------|------------------------------------|---------------------------------------|
| 0-7   | 3886           | 3506           | 380                      | 26.5                               | 140                                   |
| 7-14  | 3506           | 3062           | 444                      | 32.3                               | 167                                   |
| 14-21 | 3062           | 2557           | 505                      | 36.8                               | 188                                   |
| 21-28 | 2557           | 2388           | 169                      | 12.3                               | 64                                    |
| 28-35 | 2388           | 1768           | 620                      | 40.7                               | 208                                   |

Table 22. Heart Rate (HR) and Distance Traveled means and the 95% Confidence Interval

|                               | Horse  | HR<br>(bpm) | 95%<br>Confidence<br>Interval |    | Distance traveled<br>(km) | 95% Confidence<br>Interval |      |
|-------------------------------|--------|-------------|-------------------------------|----|---------------------------|----------------------------|------|
| Control<br>(CTRL)             | Boston | 41          | 39                            | 43 | 2.29                      | 1.98                       | 2.60 |
|                               | Uno    | 44          | 42                            | 46 | 2.08                      | 1.77                       | 2.38 |
|                               | Diego  | 43          | 41                            | 45 | 1.82                      | 1.51                       | 2.12 |
|                               | Fiji   | 43          | 41                            | 44 | 1.25                      | 0.94                       | 1.56 |
|                               | Goose  | 44          | 42                            | 46 | 1.75                      | 1.44                       | 2.06 |
| Restricted<br>Grazing<br>(RG) | Apollo | 40          | 38                            | 42 | 1.32                      | 1.02                       | 1.64 |
|                               | Vegas  | 39          | 37                            | 41 | 1.11                      | 0.80                       | 1.42 |
|                               | Whit   | 37          | 35                            | 38 | 1.35                      | 1.04                       | 1.66 |
|                               | Peri   | 32          | 30                            | 34 | 1.03                      | 0.69                       | 1.36 |
|                               | Cruz   | 40          | 38                            | 42 | 1.22                      | 0.91                       | 1.53 |



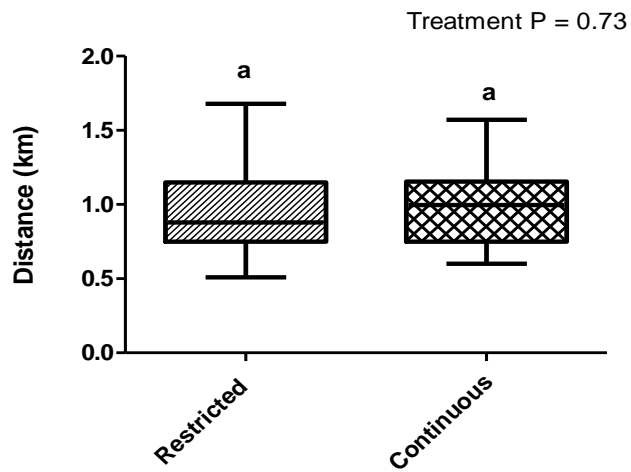
**Figure 9.** Weekly change in body weight (BW) and body condition score (BCS) from d-0 to d-35 in horses having access to time-and space-restricted grazing (RG) and those with 24-h pasture access (CTRL). Both groups were maintained on similar pasture for 35 d. The RG group lost  $35 \pm 4$  kg of BW and  $1.0 \pm 0.5$  in BCS and the CTRL group gained  $14 \pm 4$  kg of BW and  $0.5 \pm 1.0$  in BCS.



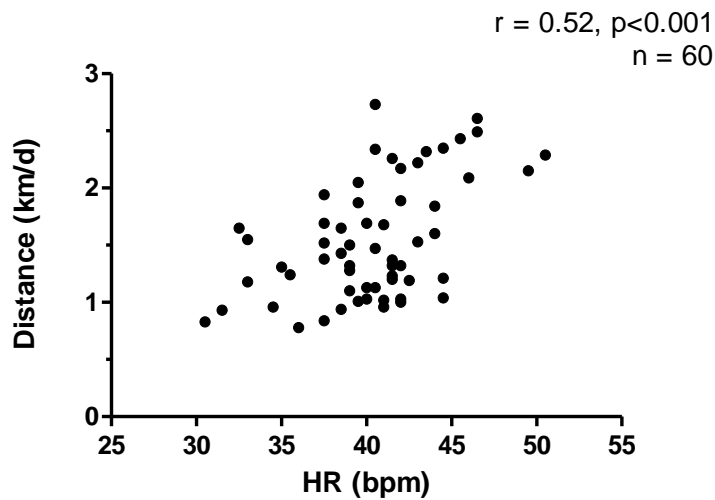
**Figure 10.** Weekly change in resting insulin concentration from d-0 to d-35 in horses having access to time- and space-restricted grazing (RG) and those with 24-h pasture access (CTRL).



**Figure 11.** These graphs represent the initial (period1) and final (period 2) activity monitoring period for RG and CTRL groups. There was a significant period within a treatment effect for HR and distance traveled. It appears that the RG group had similar HRs and traveled a similar distance to the CTRL horses by the end of the trial period.



**Figure 12.** Comparison of daytime distance traveled measurements for the restricted grazing (RG) and control (CTRL) groups there was no difference in daytime grazing (08:00 to 16:00) between the RG and CTRL groups during the trial period, after controlling for the effect of time.



**Figure 13.** Correlation between distance traveled (km/d) and average heart rate (HR) (n=60) for the ten horses (6 paired measures per horse).

## Literature Cited

- Webb, A. I., and Weaver, B. M. Q. 1979. Body Composition of the horse. *Equine Vet. J.* 11: 39-47.
- Walker, G. A., and J. Shostak. 2010. Common statistical methods for clinical research with SAS examples. SAS Institute.
- Dugdale, A. H. A., G. C. Curtis, P. Cripps, P. A. Harris, C. M. Argo. 2010. Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Vet. J.* 42(7): 600-610.
- Pratt-Phillips, S. E., Wycoff, C., Sykes, C., Kutzner-Mulligan, J., and P. D. Siciliano. 2013. Glucose and insulin concentrations in restricted pasture-fed horses. *J. Equine Vet. Sci.* 33(5): 351.
- Ungur, J., M. Blüher, M. Coenen, J. Raila, R. Boston, I. Vervuert. 2012. Effects of body weight reduction on blood adipokines and subcutaneous adipose tissue adipokine mRNA expression profiles in obese ponies. *Vet. Rec.* 171(21): 528.
- Geor, R.J. 2010. Nutrition and exercise in the management of horses and ponies at high risk for laminitis. *J. Equine Vet. Sci.* 30(9): 463-470.
- Hoffman, R.M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *J. Anim. Sci.* 81(9): 2333-2342.
- Geor, R. J. 2008. Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *J. Equine Vet. Sci.* 28(12): 753-759.
- Frank, N., R. J. Geor, S. R. Bailey, A. E. Durham, P. Johnson. 2010. Equine metabolic syndrome. *J. Vet. Intern. Med.* 24(3): 467-475.
- Argo, C. M., C. G. Curtis, D. Grove-White, A. H. A. Dugdale, C. F. Barfoot, P. A. Harris. 2012. Weight loss resistance: a further consideration for the nutritional management of obese Equidae. *Vet. J.* 194(2): 179-188.
- McGowan, C. M., A. H. A. Dugdale, G. L. Pinchbeck, C. M. Argo. 2013. Dietary restriction in combination with a nutraceutical supplement for the management of equine metabolic syndrome in horses. *Vet. J.* 196(2): 153-159.
- Van Weyenberg, S., M. Hesta, J. Buyse, G. P. J. Janssens. 2008. The effect of weight loss by energy restriction on metabolic profile and glucose tolerance in ponies. *J. Anim. Physiol. Anim. Nutr.* 92(5): 538-545.

Lawrence, L. M., Cymbaluk, N. F., D. W. Freeman, R. J. Geor, P. M. Graham-Thiers, A. C. Longland, et al. 2007. Nutrient requirements of horses. 6th ed. The National Academies Press, Washington DC.

Henneke, D., G. Potter, J. Kreider, B. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15(4): 371–372.

Pagan, J. D. 1998. Nutrient digestibility in horses. *Adv. Equ. Nutr.* 77–83.

Longland, A. C., C. Barfoot, and P. A. Harris. 2012. The effect of wearing a grazing muzzle vs. not wearing a grazing muzzle on intakes of spring, summer and autumn pastures by ponies. In: *Forages and grazing in horse nutrition*. Wageningen Academic Publishers. 185-186.

Dowler, L. E., P. D. Siciliano, S. E. Pratt-Phillips, M. Poore. 2012. Determination of pasture dry matter intake rates in different seasons and their application in grazing management. *J. Equine Vet. Sci.* 32(2): 85–92.

Reimers, T. J., R. G. Cowan, J. P. McCann, M. W. Ross. 1982. Validation of a rapid solid-phase radioimmunoassay for canine, bovine, and equine insulin. *Am. J. Vet. Res.* 43(7): 1274–1278.

Motulsky, H. J. 2007. Prism 5 statistics guide. GraphPad Software Inc, San Diego, CA.

Walker, G. A. 2002. Common statistical methods for clinical research. 2nd. SAS Institute, Inc, Cary, NC.

Chavez, S. J., P. D. Siciliano, G. B. Huntington. 2014. Intake estimation of horses grazing tall fescue (*Lolium arundinaceum*) or fed tall fescue hay. *J. Anim. Sci.* 92(5): 2304–2308.

Siciliano P. D. 2012. Estimation of pasture dry matter intake and its practical application in grazing management for horses. *Proc. 10th Mid-Atlantic Nutr. Conf.*, Timonium, MD.

Ince, J., A. C. Longland, J. C. Newbold. 2011. Changes in proportions of dry matter intakes in ponies with access to pasture and haylage for 3-20 hours per day, respectively, for 6 weeks. *J. Equine Vet. Sci.* 31(5): 283.

Dowler L. E. 2009. Prediction of hourly dry matter intake in horses; [MS Thesis] Animal Science Department at North Carolina State University, Raleigh, NC.

Frank, N., B. E. Sarah, E. B. Laura, H. K. Duane. 2006. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *J. Am. Vet. Med. Assoc.* 228(9): 1383–1390.

Geor, R. J., and P. Harris. 2009. Dietary management of obesity and insulin resistance: countering risk for laminitis. *Veterinary Clinics of North America: Equine Practice*. 25(1): 51-65.

Glunk, E. C., C. C. Sheaffer, M. R. Hathaway, and K. L. Martinson. 2014. Interaction of grazing muzzle use and grass species on forage intake of horses. *J. Equine Vet. Sci.* 34(7): 930-933.

Glunk, E. C., C. C. Sheaffer, M. R. Hathaway, and K. L. Martinson. 2013. The interaction of grazing muzzle use and grass species on forage intake of horses: a preliminary study. *J. Equine Vet. Sci.* 33(5): 357.

Siciliano, P. D. and S. Schmitt. 2012. Effect of restricted grazing on hindgut pH and fluid balance. *J. Equine Vet. Sci.* 32(9): 558-561.

Rayburn, E. B., and S. B. Rayburn. 1998. A standardized plate meter for estimating pasture mass in on-farm research trials. *Agronomy J.* 90(2): 238-241.

Bott, R.C., E. A. Greene, K. Koch, K. L. Martinson, P. D. Siciliano, C. Williams, N. L. Trottier, A. Burk, and A. Swinker. 2013. Production and environmental implications of equine grazing. *J. Equine Vet. Sci.* 33(12):1031-1043.

Glunk, E. C., and P. D. Siciliano. 2011. Effect of restricted grazing on dry matter intake rate. *J. Equine Vet. Sci.* 31(5): 296-297.

Longland, A. C., J. Ince, J. C. Newbold, C. Barfoot, and P. A. Harris. 2011. Pasture intake in ponies with and without a grazing muzzle and over time. *Proc. 15th Congr. ESVCN, Zaragoza (Spain)*: Sep, 14-16.

Duren, S. E., C. T. Dougherty, S. G. Jackson, and J. P. Baker. 1989. Modification of ingestive behavior due to exercise in yearling horses grazing orchardgrass. *Appl. Anim. Behaviour Sci.* 22(3-4): 335-345.

Collas, C., B. Dumont, R. Delagarde, W. Martin-Rosset, G. Fleurance. 2015. Energy supplementation and herbage allowance effects on daily intake in lactating mares. *J. Anim. Sci.* 93(5): 2520-2529.

Siciliano, P. D., and J. C. Gill. 2015. Effect of pasture sward height on blood glucose and insulin profiles in grazing horses. *J. Equine Vet. Sci.* 35(5): 429.

Krzak, W. E., H. W. Gonyou, and L. M. Lawrence. 1991. Wood chewing by stabled horses: diurnal pattern and effects of exercise. *J. Anim. Sci.* 69(3): 1053-1058.

Waters, A. J., C. J. Nicol, and N. P. French. 2002. Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study. *Equine Vet. J.* 34(6): 572-579.

Hothersall, B., and C. Nicol. 2009. Role of diet and feeding in normal and stereotypic behaviors in horses. *Veterinary Clinics of North America: Equine Practice* 25(1): 167-181.

Green, P., and J. M. Tong, J. M. 1988. Small intestinal obstruction associated with wood chewing in two horses. *The Veterinary Record* 123(8): 196-198.

Curtis, G. C., C. F. Barfoot, A. H. Dugdale, P. A. Harris, and C. M. Argo. 2011. Voluntary ingestion of wood shavings by obese horses under dietary restriction. *British J. Nutr.* 106(S1): S178-S182.

Reeves, M. J., M. D. Salman, and G. Smith. 1996. Risk factors for equine acute abdominal disease (colic): results from a multi-center case-control study. *Prev. Vet. Med.* 26(3): 285-301.

Gentry, L. R., D. L. Thompson, G. T. Gentry, K. A. Davis, R. A. Godke, J. A. Cartmill. 2002. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J. Anim. Sci.* 80(10): 2695-2703.

Pratt, S. E., P. D. Siciliano, and L. Walston. 2009. Variation of insulin sensitivity estimates in horses. *J. Equine Vet. Sci.* 29(6): 507-512.

Rogalski M. 1975. Effect of weather conditions and grazing management on the behavior of horses on pasture. *Rocz.-Nauk-Roln. Ser-B. Zootech* 97: 7-16.

Cantillon, J. D. 1986. Measurement of intake levels and examination of grazing parameters of Quarter Horse geldings. M.S. Thesis, University of Kentucky, Lexington.

## GENERAL CONCLUSIONS

Weight management in horses is important in maintain a healthy body weight. Understanding variability in calorie requirements and energy expenditure are important when determining feeding rate in horses. These factors may affect the efficiency of weight loss or weight gain in horses. The inability of owners to properly maintain body condition in horses and ponies has led to the increased incidence in obesity seen in the US and UK. Overweight horses have an increased risk of insulin resistance and laminitis. Overweight horses undergoing treatment for laminitis are recommended for weight loss management by their owners. Weight loss in obese horse has been well documented in a controlled weight loss setting, but little research has investigated the capacity of owners to carry out similar protocols at private facilities. EXPT1 was conducted to determine if 10% to 20% calorie restriction could be carried out successfully in overweight horses that were managed by their owners. It was concluded that dietary restriction can be implemented successfully in horses when managed by their owners; however the greatest rate of weight loss was observed in horses with the most compliant owners and in the absence of pasture. Variability in weight loss between horses with even very compliant owners in the absence of pasture was possibly related to differences in breed, age, body composition, voluntary activity and basal metabolism. EXPT2 was conducted to evaluate the relationships among digestible energy (DE) intake, body weight, and body condition in lean/moderate condition horses fed above maintenance intakes. The results confirmed that horse-horse variability exists in confined horses consuming similar intakes, possibly arising

from differences in basal metabolism and voluntary activity. Overall, horses required  $24 \pm 3$  Mcal per kg BW gain, which was consistent with the current literature. EXPT3 was conducted to present novel findings regarding the within-horse variability associated with activity (HR and distance traveled) and pasture DMI in horses. Activity in grazing horses and variability in model parameters could provide insight into factors affecting energy balance in horses. Heart rate has been associated with energy expenditure in exercising horses, whereas fecal output is positively associated with DMI. It was found that HR and fecal output were repeatable measures, which showed good method performance. Variation in activity-associated parameters between horses may arise from differences in metabolism and pasture activity between horses. The horse with the highest mean HR was the most obese, indicating the possibility of heat stress. Results showed horses were not consistent in their grazing activity across days of study. A future study assessing the differences in activity between horses should include more days of distance traveled collection and an observation of individual horse activities at pasture.

Grazing is an important aspect of nutrition for horses, but unlimited pasture access can result in the overconsumption of forage by horses leading to obesity. Weight loss studies in horses are normally conducted in the absence of pasture because of limited methods of pasture restriction and the inability to accurately determine calories from pasture in horses. However, pasture grazing is an important aspect of equine nutrition and turnout represents a source of potential activity and socialization to aid in weight management. In addition, some husbandry situations do not support the complete removal of horses from pasture for weight loss. EXPT4 investigated the effect

of time-and space-restricted grazing result in a reduction in BW, BCS and INS, while promoting complete and uniform removal of forage. Ultimately, restricting grazing in horses by reducing the time at pasture and the space allocated for grazing (herbage allowance) can successfully reduce BW and BCS, although does not result in a significant improvement in resting INS concentration. Resting INS concentration appeared to mirror the changes in pasture NFC content and it is possible that transitioning repetitively from sparse to lush pasture (when utilizing a strip grazing system) may negatively impact INS and hindgut fermentation parameters. Overall, weight management in horses can be successfully implemented through restricted pasture access.

## APPENDIX

Table 23. Estimated dry matter intake (DMI) using the pasture dry matter digestibility (DMD) range of 55% and 60%.

| Horse | eDMI <sup>1</sup><br>(g DM·kg BW <sup>-1</sup> · d <sup>-1</sup> ) | eDMI <sup>1</sup><br>(kg/d) | eDMI <sup>2</sup><br>(g DM·kg BW <sup>-1</sup> · d <sup>-1</sup> ) | eDMI <sup>2</sup><br>(kg/d) |
|-------|--|-----------------------------|--|-----------------------------|
| 1     | 18.34 ± 0.86   | 10.67 ± 0.48                | 20.63 ± 0.93   | 12.00 ± 0.53                |
| 2     | 18.36 ± 0.53   | 12.54 ± 1.49                | 20.66 ± 2.63   | 14.11 ± 1.67                |
| 3     | 16.94 ± 1.34   | 8.81 ± 0.70                 | 18.86 ± 1.79   | 9.91 ± 0.78                 |
| 4     | 19.72 ± 1.27   | 11.54 ± 0.74                | 23.25 ± 0.26   | 12.98 ± 0.83                |
| 5     | 18.13 ± 0.48   | 12.04 ± 0.32                | 20.27 ± 0.60   | 13.54 ± 0.35                |
| 6     | 16.51 ± 1.25   | 9.71 ± 0.73                 | 18.87 ± 1.99   | 10.92 ± 0.82                |
| 7     | 15.03 ± 2.02   | 10.26 ± 1.36                | 16.91 ± 2.27   | 11.55 ± 1.53                |
| 8     | 26.97 ± 2.16   | 15.39 ± 1.01                | 30.34 ± 2.43   | 17.315 ± 1.13               |

<sup>1</sup> DMD estimated at 55%

<sup>2</sup> DMD estimated at 60%

DMI estimates are the mean ± SD of 6 daily measures.

Table 24. Estimated digestible energy intake (DEI) using pasture dry matter digestibility (DMD) of 55% and 60%.

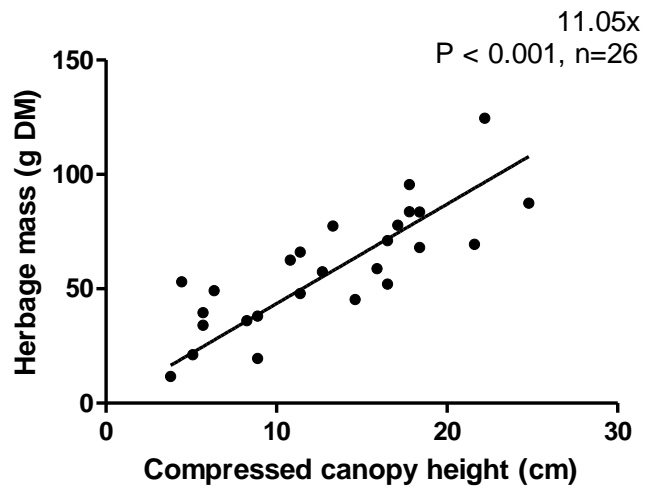
| Horse | DEI <sup>1</sup><br>(Kcal·kg BW <sup>-1</sup> ·d <sup>-1</sup> ) | DEI <sup>2</sup><br>(Kcal·kg BW <sup>-1</sup> ·d <sup>-1</sup> ) |
|-------|--|--|
| 1     | 41.3   | 46.4   |
| 2     | 41.3   | 46.4   |
| 3     | 38.1   | 42.9   |
| 4     | 44.4   | 49.9   |
| 5     | 40.8   | 45.9   |
| 6     | 37.1   | 41.8   |
| 7     | 33.8   | 38.1   |
| 8     | 60.7   | 68.3   |

<sup>1</sup> DMD estimated at 55%

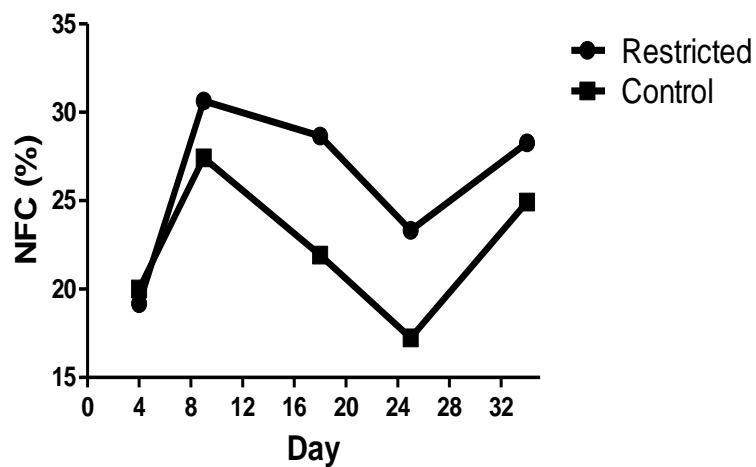
<sup>2</sup> DMD estimated at 60%

Pasture DE: 2.25 Mcal/kg

DEI estimates are the mean ± SD of 6 daily measures.



**Figure14.** Estimated herbage mass (g DM) from harvested samples according to the compressed canopy height determined from 26 compressed canopy height measurements.



**Figure 15.** Pasture non-fiber carbohydrate (NFC) content from pasture sample collection on 4, 9, 18, 25, and 34 d.

```

data horse;
input horse $ treatment $ time BCS BW;
datalines;

```

|   |    |    |     |     |
|---|----|----|-----|-----|
| 5 | EX | 0  | 5   | 480 |
| 5 | EX | 7  | 5   | 464 |
| 5 | EX | 14 | 5   | 461 |
| 5 | EX | 21 | 4.5 | 459 |
| 5 | EX | 28 | 4.5 | 457 |
| 5 | EX | 35 | 4   | 451 |
| 8 | EX | 0  | 5   | 575 |
| 8 | EX | 7  | 4.5 | 561 |
| 8 | EX | 14 | 4   | 557 |
| 8 | EX | 21 | 4   | 550 |
| 8 | EX | 28 | 3.5 | 543 |
| 8 | EX | 35 | 4   | 536 |
| 2 | EX | 0  | 8   | 634 |
| 2 | EX | 7  | 8   | 614 |
| 2 | EX | 14 | 8   | 618 |
| 2 | EX | 21 | 8   | 618 |
| 2 | EX | 28 | 7.5 | 595 |
| 2 | EX | 35 | 6.5 | 595 |
| 6 | EX | 0  | 5   | 568 |
| 6 | EX | 7  | 5   | 552 |
| 6 | EX | 14 | 5   | 552 |
| 6 | EX | 21 | 4.5 | 552 |
| 6 | EX | 28 | 4.5 | 543 |
| 6 | EX | 35 | 4   | 534 |
| 7 | EX | 0  | 8   | 661 |
| 7 | EX | 7  | 8   | 643 |
| 7 | EX | 14 | 7.5 | 643 |
| 7 | EX | 21 | 8   | 634 |
| 7 | EX | 28 | 7.5 | 632 |
| 7 | EX | 35 | 7   | 627 |
| 4 | CO | 0  | 7   | 540 |
| 4 | CO | 7  | 7   | 555 |
| 4 | CO | 14 | 7.5 | 564 |
| 4 | CO | 21 | 8   | 561 |
| 4 | CO | 28 | 7.5 | 561 |
| 4 | CO | 35 | 7.5 | 555 |
| 9 | CO | 0  | 6.5 | 605 |
| 9 | CO | 7  | 7   | 607 |
| 9 | CO | 14 | 7   | 621 |
| 9 | CO | 21 | 7.5 | 618 |
| 9 | CO | 28 | 7.5 | 612 |
| 9 | CO | 35 | 7.5 | 616 |
| 9 | CO | 0  | 4.5 | 505 |
| 3 | CO | 7  | 4.5 | 502 |
| 3 | CO | 14 | 4.5 | 516 |
| 3 | CO | 21 | 5   | 511 |
| 3 | CO | 28 | 5   | 514 |
| 3 | CO | 35 | 5   | 509 |
| 1 | CO | 0  | 6   | 575 |

|    |    |    |     |     |
|----|----|----|-----|-----|
| 1  | CO | 7  | 7.5 | 582 |
| 1  | CO | 14 | 7.5 | 586 |
| 1  | CO | 21 | 7.5 | 584 |
| 1  | CO | 28 | 7.5 | 587 |
| 1  | CO | 35 | 8   | 595 |
| 10 | CO | 0  | 7.5 | 632 |
| 10 | CO | 7  | 7.5 | 648 |
| 10 | CO | 14 | 7   | 657 |
| 10 | CO | 21 | 7   | 654 |
| 10 | CO | 28 | 7   | 652 |
| 10 | CO | 35 | 7   | 655 |

```
run;
```

```
Proc Print data=horse;
```

```
run;
```

```
proc mixed data=horse;
```

```
class treatment time horse;
```

```
model BW = treatment time time*treatment;
```

```
random horse;
```

```
Repeated / Type=AR(1) Subject=horse;
```

```
Lsmeans treatment time treatment*time/ pdiff=ALL;
```

```
run;
```

```
proc mixed data=horse;
```

```
class treatment time horse;
```

```
model BCS = treatment time time*treatment;
```

```
random horse;
```

```
Repeated / Type=AR(1) Subject=horse;
```

```
Lsmeans treatment time treatment*time/ pdiff=ALL;
```

```
run;
```

**Figure 16.** SAS input for determining the effects of horse, treatment group, and time on body weight (BW) and body condition score (BCS).