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# The Effect of Forage Quality on Voluntary Hay Intake, Serum Glucose and Insulin, Muscle Glycogen, Whole Blood Lactate, Heart Rate, and Respiratory Parameters of Exercised Horses

Caitlin M. Chase

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By

Caitlin M. Chase

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By

Caitlin M. Chase

Approved:

_____
Brian J. Rude
(Major Professor)

_____
Clay A. Cavinder
(Committee Member)

_____
Derris Devost-Burnett
(Committee Member)

_____
Jamie Larson
(Graduate Coordinator)

_____
John Blanton, Jr.
(Department Head)

_____
George Hopper
(Dean)
College of Agriculture and Life Sciences

Name: Caitlin M. Chase

Date of Degree: August 10, 2018

Institution: Mississippi State University

Major Field: Animal Nutrition

Major Professor: Brian J. Rude

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Candidate for Degree of Master of Science

Six mares and six geldings were used to determine the effect of forage quality on muscle glycogen utilization by exercised horses. Horses were fed 1.5% BW/d of high quality (65.6% NDF; 41.1% ADF) or low quality (74.6% NDF; 51.2% ADF) hay and a concentrate resulting in three diets: high quality hay and concentrate balanced to meet energy requirements (HQ); low quality hay and concentrate balanced to meet energy requirements; and low quality hay with the same amount of concentrate as the HQ diet. The effect of hay quality on serum insulin, serum glucose, whole blood lactate, and respiratory parameters were also determined. Results suggested varied metabolic responses to exercise between sexes and a possible glycogen sparing effect when low quality forage was consumed.

# DEDICATION

I would like to dedicate this work to my exceptional parents, Christopher and Reece Chase. I doubt you expected that after my first riding lesson that I would go on to seek a degree relating to horses, but thank you so much for humoring that horse-crazed little girl. You have been my dedicated and unwavering supporters throughout this endeavor, and I cannot express the depth of my love and gratitude to you.

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# CHAPTER I

# LITERATURE REVIEW

## 1.1 Equine Muscle

As with other animal species, there are 3 basic types of muscle within the equine body: smooth, cardiac, and skeletal. Of particular importance and interest in exercising horses is skeletal muscle. Skeletal muscle is comprised of many muscle bundles, which are in turn comprised of many muscle fibers. Individual muscle fibers are, in fact, single elongated cells which are nourished by an extensive capillary network within the muscle (Marlin and Nankervis, 2002). Initially, muscle fibers were classified based upon their color and contractile speeds as red, tonic fibers or white, tetanic fibers (Beecher et al., 1965). Muscle fibers can be further classified into type I, type IIA, and type IIB fibers based on their size, histochemical staining pattern, and oxidative capacity, which is dependent on myosin ATPase activity (Baldwin et al., 1973). This method of classification was suggested by Brooke and Kaiser (1970) in order to eliminate discrepancies resulting from inconsistent terminology in research. Histochemical staining patterns vary depending on the acidity or alkalinity of the stain utilized (Marlin and Nankervis, 2002; Hodgson et al., 2014), but Snow and Guy (1980) reported that, at a pH of 4.3, red muscle fibers resulted in a dark stain and low or intermediate staining resulted from white muscle fibers. More recent methods for muscle fiber type identification include immunostaining which utilizes antibodies specific to myosin heavy chain (MHC)

isoforms distinct for each fiber type (Sawano et al., 2016). Using methods distinguishing fiber types based on MHC isoforms, a third type II fiber, type IIX, has been distinguished (Schiaffino et al., 1989). The majority of existing literature concerning equine fiber types still distinguishes muscle fibers primarily into type I, type IIA, and type IIB.

Type I fibers have the greatest capacity for aerobic energy production, and so contract more slowly than both type II fibers (Valberg et al., 1985). Additionally, they are generally narrower in diameter than type II fibers (Ashmore et al., 1972), and so are not capable of generating as much power. Mitochondria are profusely present in type I fibers, which makes them ideally suited to function during prolonged, sub-maximal exercise (Beecher et al., 1965; Snow and Guy, 1976). Lipid content is greater and glycogen content is lower in type I fibers compared to type II fibers, and type IIA fibers have greater lipid content that do type IIB fibers (Valberg et al., 1985).

Type IIA and B fibers have less oxidative capacity than type I fibers. Type IIA fibers are considered intermediate fibers between types I and IIB, because they are nearly equally suited to both aerobic and anaerobic energy production (Valberg et al., 1985). They are capable of contractile speeds greater than that of type I fibers and are generally larger in diameter (Ashmore et al., 1972; Armstrong and Phelps, 1984) and so capable of generating more power. Type IIB fibers are genuinely anaerobic fibers and have very low oxidative capacity, but they are capable of the greatest contractile speed and have a large glycolytic capacity (Marlin and Nankervis, 2002). Due to the reduced oxidative capacity and need for oxygen supply, Type IIB fibers generally do not exhibit the same, extensive capillary presence as Types I and IIA (Marlin and Nankervis, 2002).

Fiber types are relied upon more heavily during exercise, or recruited, according to their oxidative capacity and the intensity of exercise (Marlin and Nankervis, 2002). Generally, type I and then IIA are recruited first at low speeds and to maintain posture. Type IIB are recruited at higher speeds, during rapid acceleration, and during jumping (Yamano et al., 2011; Marlin and Nankervis, 2002). It is important to note, that during prolonged submaximal exercise, such as an endurance event, Types I and IIA may begin to run out of glycogen and so type IIB fibers would be more heavily recruited (Marlin and Nankervis, 2002).

The prevalence of particular muscle fiber types found in an individual is genetically determined. Horses with a greater percentage of type I fibers will naturally have greater stamina, while those with a high percentage of type II fibers will have better sprinting ability (Marlin and Nankervis, 2002). This has been well documented in elite human athletes (Snow and Guy, 1980; Tesch and Karlsson, 1985, Metaxas et al., 2014). The hindquarter muscles of horses generally have a large prevalence of type II fibers, meaning that they have natural propensity for sprinting and generating locomotive force during exercise. The stamina and endurance of horses can be increased with training (Lindholm and Peihl, 1974).

Variations in the prevalence of fiber types exist between breeds as well, and so, to some degree, muscle fiber characteristics can determine a breed's and individual horse's suitability for a particular discipline (Marlin and Nankervis, 2002). Heavier muscled horses, such as stock-type breeds, typically have a greater proportion of IIB compared to IIA fibers (Ashmore et al., 1972). Variations also exist within particular muscle groups of an individual horse. Muscles in a horse's forelimb generally have a greater percentage of

type I fibers, indicating their role in maintaining posture and supporting the horse's weight during exercise (Marlin and Nankervis, 2002). The pattern of variation continues even within an individual muscle. Type II fibers are generally more concentrated on the outside of the muscle, while type I fibers are more concentrated in the muscle belly (Marlin and Nankervis, 2002). López-Rivero and colleagues found that both type I and IIA fibers located in deeper regions of the muscle tend to be larger in diameter than in superficial areas, and that IIB fibers deep within the *gluteus medius* are typically smaller in diameter than in superficial areas (1991). The same study found that type I fibers are larger than both type II fibers deep in the muscle, and the size of I fibers is more homogenous deep in the muscle. Type IIB fiber size also varied more in superficial areas of the muscle. Greater force is able to be exerted by having more powerful fibers located on the outside of the muscle, because the muscle is able to be shortened more efficiently than if the more powerful fibers were located on the inside of the muscle (Marlin and Nankervis, 2002).

Differences of fiber type prevalence also exist between sexes. Stallions generally have a larger concentration of type IIA fibers, which indicates that they are more capable of both power and stamina (Marlin and Nankervis, 2002). As horses age, the number of type IIA fibers increases, generally at the expense of type IIB fibers (Seeherman and Morris., 1991). However, the percentage of type IIA fibers is greater in stallions compared to mares. Even within breed, variation in muscle fiber composition exists between individual horses. Additionally, muscle fiber composition can be altered with training for a particular discipline. Generally, as a horse is introduced to a training program, aerobic capacity of the horse increases, accompanied by additional increases in

exercise capacity and endurance (Seeherman and Morris, 1991). This is due to increased oxidative capacity of type IIB fibers. In fact, Type IIB fibers may begin to appear as type IIA fibers when stained as a horse becomes more aerobically fit (Marlin and Nankervis, 2002).

## 1.2 Equine Skeletal Muscle Glycogen

Compared to human athletes, horses have a vast capacity to store glycogen in skeletal muscle. The minimum equine glycogen unit is considered a chain of 12 glucopyranose units linked by 1:4- $\alpha$ -glycosidic links (Bell, 1937). Glycogen concentrations between 500 and 650 glucosyl units per kg of dry weight are common in equine skeletal muscle, compared to 300 to 400 glucosyl units per kg of dry weight commonly found in humans (Snow and Harris, 1991). Training can increase an individual's ability to store glycogen in skeletal muscle (Piehl et al., 1974; Ren et al., 1994).

Glycogen is present to some degree in all muscle fiber types, though magnitude of storage of glycogen in a particular fiber type is dictated by the primary modes of energy production within that fiber type (Baldwin et al., 1973). During postnatal development a decrease of mitochondrial density of muscle fibers is accompanied by an increase of glycogen phosphorylase, which is an indicator of the prevalence of glycolytic enzymes in general (Ashmore et al., 1972). Hodgson et al. (1984) found that type IIA and IIB fibers of Thoroughbreds competing in 800 and 1200 m barrier races stored more glycogen than did type I fibers. A similar study by Valberg and colleagues (1985) found that horses which exhibited a greater percentage of type IIA fibers utilized less glycogen than horses with a greater percentage of type IIB fibers. The researchers in this study concluded that

horses with greater percentages of fiber types I and IIA likely are able to spare the use of glycogen either via the oxidation of pyruvate or the utilization of alternative substrates, such as triglycerides, free fatty acids, or glucose from the bloodstream. Type IIB fibers store and utilize the greatest amount of glycogen. For this reason, it is important to carefully select which muscle is most appropriate to use for muscle biopsy to determine glycolytic response to stimuli. The *biceps femoris*, has been found to have relatively little variation in fiber type and a large prevalence of Type IIB fibers.

Snow and others (1985) regularly exercised 6 horses over the course of 2 months, and the horses then performed a standardized exercise test comprised of four consecutive 620 m maximal gallops at the end of the second month. The investigators concluded that the mean rate of glycogen breakdown throughout the exercise test was 1.08 ± 0.21 mmol glucosyl units • kg dry matter$^{-1}$ • s$^{-1}$. Humans typically have comparatively less rate of glycogen utilization during maximal exercise of 0.72 mmol glucosyl units • kg dry matter$^{-1}$ • s$^{-1}$ (Saltin and Karlsson, 1971). Similarly, glycogen depletions of 167 ± 22.2 mmol and 158.2 ± 23.2 mmol glucose units • kg dry weight$^{-1}$ were observed in Thoroughbreds competing in 800 and 1200 m barrier races, respectively (Hodgson et al., 2006). In this study, only Types IIA and IIB displayed glycogen depletion, which reflects the anaerobic activity of these fibers during maximal exercise.

Several studies have investigated the effect of warm-up on energy substrate metabolism in skeletal muscle during exercise. In a study by McCutcheon et al. (1999), 6 horses underwent an exercise test following no warm-up, low intensity warm-up, and high intensity warm-up in a crossover study. The researchers found that while warm-up of either intensity resulted in less muscle glycogen concentration, following both the

warm-up period and the exercise test compared to no warm-up, when expressed as a function of run time muscle glycogen degradation was 40% less and 20% less than that of no warm-up for low intensity and high intensity warm up, respectively. These results suggest that warm-up prior to exercise results in increased efficiency of energy substrate metabolism within skeletal muscle during exercise.

## 1.3 Metabolism

As with any athlete, the equine has a remarkable capacity for energy production via both aerobic and anaerobic energy production. Oxygen is necessary for aerobic energy production, and when predominately aerobic metabolism is utilized, type I fibers are recruited before type II fibers. Aerobic metabolism is utilized primarily during sustained, submaximal exercise, when oxygen supply to muscle is not as limited as it would be during maximal exercise. When exercise intensity is below 50 % of the maximal oxygen uptake capacity for a particular horse, aerobic metabolism is utilized (Wasserman and Whipp, 1975). As anaerobic metabolism is not reliant upon oxygen in order to produce ATP, it is often utilized during short, maximal exercise. Type II fibers are more heavily recruited when anaerobic metabolism occurs. Exercise intensities above 50 % of the maximal oxygen uptake capacity begin to recruit anaerobic metabolism, when energy demands of exercise exceeds the capacity of oxidative pathways (Wasserman and Whipp, 1975).

The glycolytic pathway, which is responsible for oxidizing organic molecules to produce ATP, is the first step for both anaerobic and aerobic metabolism. Carbohydrates are the primary component of the equine diet (Pagan, 1998), and, of carbohydrates, glucose is the most abundant energy source available for both animals and plants. During

aerobic metabolism, pyruvate produced from glycolysis is converted to acetyl-coenzyme A (acetyl-CoA) by pyruvate dehydrogenase complex. Acetyl-CoA then enters the citric acid cycle for energy production via oxidative phosphorylation. During aerobic metabolism, 36 ATP can be produced from a single glucose molecule.

Lactate is produced when tissues cannot be supplied with adequate amounts of oxygen to facilitate aerobic oxidation of pyruvate and NADH, both of which are produced in the glycolytic pathway. Nicotinamide adenine dinucleotide is regenerated anaerobically from NADH via the reduction of pyruvate to lactate instead, a reaction catalyzed by lactate dehydrogenase. In extremely strenuous exercise, the lactate produced in skeletal muscle is then transported to the liver by blood where it can used for gluconeogenesis during recovery from exercise via the Cori Cycle. Glucose produced from gluconeogenesis at this time can be used to begin to replenish skeletal muscle glycogen depots.

Blood lactate is a commonly used indication of glycolytic activity, oxygen consumption, individual fitness, and exercise intensity across multiple species. Valberg and colleagues (1985) found a positive correlation between glycogen utilization in horses and both lactate accumulation in the muscle and concentration in the blood. Horses have an exceptional ability to accumulate lactate. Both humans and horses have similar resting lactate concentrations at roughly 1 mmol/L, however peak lactate in horses can reach concentrations twice that typical of humans at 30 compared to 15 mmol/L (Snow and Mackenzie, 1977).

Onset of blood lactate accumulation is commonly used in equine exercise physiology as a determinant of fitness or exercise intensity and is understood to be the

velocity at which blood lactate reaches 4 mmol/L (Wasserman and Whipp, 1975; Marlin and Nankervis, 2002). Onset of blood lactate is often used in conjunction with "anaerobic threshold." Anaerobic threshold refers to the intensity of exercise at which blood lactate begins to accumulate more rapidly (Marlin and Nankervis, 2002) but can be misleading in that it implies that energy production at exercise intensities meeting or exceeding the anaerobic threshold is completely anaerobic resulting in lactate production. This is not the case, and in fact, energy production during exercise is not entirely aerobic or anaerobic. Anaerobic threshold instead refers to the notion that anaerobic pathways will be called upon gradually and relied upon more heavily as exercise intensity increases. During instances of vigorous anaerobic exercise, lactate can be produced in large quantities, and can limit the amount of time during which intensely anaerobic exercise can occur (Marlin and Nankervis, 2002). Lactate that is produced in the skeletal muscle rather rapidly dissociates into lactate ions and hydrogen ions (Marlin and Nankervis, 2002). Although lactate will then form a salt with either sodium or potassium ions, hydrogen ions can rapidly accumulate and result in a more acidic muscular environment (Marlin and Nankervis, 2002). Accumulation of lactate can lead to a decrease in the pH of active muscles and therefore reduce muscle efficiency.

Results of studies investigating the relationship between speed of exercise and lactate response have been inconsistent. Some studies report a correlation between lactate accumulation and performance in a timed event (Evans et al., 1993). However, it has become apparent that with increased training and fitness, individuals become more tolerant of lactate and are able to perform near maximal exercise for longer periods of time, which is made apparent by the relatively unchanged lactate concentrations present

in trained individuals, both horses and humans. Training results in increased capillary diffusion per unit of tissue, blood clearance rate, and the muscle to blood gradient (Hollozy, 1967; Harris et al., 1987). Robinson and Harmon in 1941 found there was no change in basal lactate concentration when distance runners performed submaximal exercise. Subjects were capable of withstanding greater lactate concentrations during maximal exercise, but a decline in lactate concentration during exercise was found. This result suggests that circulation and oxygen supply to leg muscles improved with training, and that lactate was able to be distributed throughout the body for increased buffering. When comparing trained and untrained horses at an identical workload, it has been observed that trained individuals have a more rapid return to resting lactate concentrations (Snow and Mackenzie, 1977). This has been attributed to an increase in uptake and metabolism of lactate by tissues associated with increased efficiency of oxygen uptake. Further alterations in metabolism as a result of training include an increase in hexokinase, a major glycolytic enzyme, activity in all fiber types (Baldwin et al., 1973). These researchers found that hexokinase activity increased 170 % in red muscle and 30 % in white muscle in trained rats. It is important to note that in this study, the exercise underwent by the rats likely was not strenuous enough to result in frequent contraction of white muscle fibers. This lack of stimulation could have been the reason that hexokinase activity was not further increased.

## 1.4 Starch Digestion in the Horse

As hindgut or post-gastric fermenters, horses are able to utilize products of fiber fermentation, which occurs in the cecum. The products of fermentation in the hindgut are capable of sustaining most idle horses, however, supplementation with energy-dense

concentrate may be required when energy demands of a horse are increased due to exercise. Starch is a large component of many concentrate feeds and is composed of glucose units with α-1,4 linkages. While fiber digestion occurs primarily in the hindgut of the horse, the bulk of starch digestion occurs in the foregut. The foregut of the horse is very similar to that of monogastric animals. Components of the foregut include the esophagus, stomach, duodenum, jejunum, and ilium. Digestion begins with mastication in the mouth, followed by deglutition. Very little salivary amylase is produced by the horse, so starch digestion occurs only minimally until digesta reaches the small intestine (Hoffman, 2009). Once the digesta enters the small intestine from the stomach, pancreatic amylase begins to hydrolyze α-1,4 linkages in nonstructural carbohydrates, and most absorption of the resulting glucose occurs in the small intestine as well (Hoffman, 2009).

Though supplementation with high-starch concentrates can be beneficial for meeting increased energy demands, starch digestion that begins to occur in the hindgut can be extremely problematic (Kienzle et al., 1992; Kienzle, 1994), resulting in issues such as colic and laminitis. Feeding starches with greater preileal digestibility and relatively slow rate of fermentation in the cecum logically are less likely to result in cecal dysfunction than those starch types which are not readily digested preileally but are rapidly fermented in the cecum (Kienzle et al., 1997).

Within insulin-sensitive tissue, glucose uptake from the blood stream has proven the rate-limiting step in all species, and skeletal muscle is among the tissues which utilize the most glucose as an energy substrate (Lacombe, 2014). The lipid bilayers of cell membranes are impermeable to glucose, and so most cells must uptake glucose by utilizing glucose transporters (GLUTs), which are integral membrane proteins (Mueckler

and Thorens, 2013). Epithelial cells of small intestine's brush border absorb glucose actively by using a $Na^+$/glucose cotransporters to transport glucose against the electrochemical gradient (Zhao and Keating, 2007). The capacity of equine intestinal $Na^+$/glucose cotransporter and its expression has been found to increase with increased dietary carbohydrate consumption (Dyer et al., 2009).

Despite increased absorptive capacity, starch digestive capacity of horses has been found to decrease with increased starch intake (Kienzle et al., 1997). Furthermore, starch that is not digested in the foregut will enter the hindgut, which can lead to digestive upset, as mentioned previously. Digestibility of feedstuffs is known to vary, especially due to differences in mechanical treatment and processing prior to feeding. Mechanical treatment can alter starch structures of both the grain and starch granules without affecting starch solubility, though both breaking and grinding grains increases the digestibility of the concentrate (Kienzle et al., 1997). Another study by Kienzle (1988) found that liberation of maltose from soluble starch by digestion with pancreatic amylase from horses, pigs, dogs, and cats was between 10 and 100 times faster than from unprocessed, insoluble starch. Differences in mastication behaviors between horses have also been determined to affect starch digestibility, as those that spent less time chewing and swallowed more quickly were found to have markedly larger sized particles and decreased digestibility of whole corn in jejunal chyme compared to horses that spent greater time chewing (Kienzle et al., 1997).

## 1.5 Fiber Digestion in the Horse

Fiber has long been understood to be an integral component of the equine diet in order to maintain proper and ideal gut function and to minimize the development of

stereotypies. Horses evolved as grazers, and their ingestive behavior and gastrointestinal tract makes them well-suited to consuming largely fibrous feeds. The hindgut is composed of the cecum, large intestine, and rectum. As hindgut fermenters, horses house a diverse population of microflora in the hindgut which is remarkably uniform among horses (Ericsson et al., 2016). This microbial population allows fiber fermentation to take place within the hindgut of the horse during which VFA are produced (Ford and Simmons, 1985). The three primary VFA are propionate, acetate, and butyrate. A substantial amount of the total energy utilized by horses is derived from metabolism of VFAs. A study by Glinsky and colleagues (1976) found that roughly 30 % of the DE intake in cecally cannulated ponies was supplied by VFA metabolism.

Relative production of the various VFAs can be influenced by diet in equines, similarly to ruminant animals. A diet in which fiber is present in greater quantities than concentrate feedstuffs will result in greater production of acetate compared to propionate and butyrate (Glinsky et al., 1976; Willard et al, 1977). Conversely, as the concentrate portion of the diet increases, propionate production will also increase and cecal pH decreases (Glinsky et al., 1976; Willard et al, 1977). Butyrate production, however, remains relatively unchanged despite manipulation of fiber and concentrate portion. Similar studies in ruminant animals have suggested that this observed difference in the VFAs produced from varying diets are the result of altered microbial populations in the rumen (Hungate, 1966). Propionate is the VFA primarily used for gluconeogenesis in both ruminants and horses, and research by Ford and Simmons (1984) sought to determine the contribution of propionate to gluconeogenesis relative to total body glucose. Cecally and colonically cannulated ponies were fed one of two diets consisting

of either chopped hay or chopped hay and wheat bran. It was determined that ponies fed chopped hay alone produced 19.6 mg propionate • $h^{-1}$ • kg body $weight^{-1}$ resulting in production of 1.7 mg glucose • $min^{-1}$ • kg $BW^{-1}$, and that ponies fed chopped hay and wheat bran produced 34.0 mg propionate • $h^{-1}$ • kg $BW^{-1}$ resulting in production of 2.2 mg glucose • $min^{-1}$ • kg $BW^{-1}$. The researchers concluded that propionate contributed roughly 7 % of total glucose in the body via gluconeogenesis.

Forages that are less mature are generally more digestible by horses than more mature forages (Müller, 2012). Generally, lignin content increases with crop maturity, which contributes to decreased NDF digestibility (Ball et al., 2007). Horses consuming more mature forages must have a greater intake in order to meet nutritional requirements due to decreased digestibility (Müller, 2012). The need for increased intake of more mature forage is problematic, however, because voluntary intake often declines with increased crop maturity by ruminant animals as the rumen has a finite capacity. Most research concerning the relationship between forage maturity and intake has found that voluntary intake of horses does not necessarily decline dramatically with crop maturity (Edouard et al., 2008), and this has been attributed to the fact that passage through the digestive tract of the equine is not as heavily dictated by particle size as that of ruminant animals (Janis, 1976; Edouard et al., 2008).

## 1.6 Glycogen Depleting Exercise

During vigorous exercise, muscle glycogen is the primary energy source for glycolysis in both humans and horses. When horses undergo repeated bouts of maximal exercise or prolonged periods of exercise with intermittent sprints, muscle glycogen may be depleted by as much as 50 % (Davie et al., 1996; Harris et al., 1987; Hodgson et al.,

1984; Lacombe et al., 1999; Snow and Harris, 1991; Snow et al., 1985). Resting muscle glycogen concentrations vary depending upon breed, athletic fitness, the muscle being biopsied and BCS, and it is important to note that not only can degree of depletion indicate the intensity of a particular exercise bout but also the degree to which a particular muscle is involved in a given exercise. Davie and colleagues (1996) reported 608 ± 53.8 mmol glycogen/kg dry weight units in race-trained Thoroughbreds, and a similar concentration of 606 to 667 mmol/kg glycosyl units was found by Snow and Harris (1991). Standardbred Trotters were found to have resting muscle glycogen concentrations ranging between 557 to 730 mmol glycogen/kg wet weight (Valberg, 1986). Oldham and others (1990) found that mares fed a fat-supplemented diet had increased resting muscle glycogen of 22.89 mg glycogen/g wet tissue compared to 15.77 in control fed mares. Another study concerning the effects of fat supplementation on muscle glycogen storage and utilization found that ponies with greater BCS had greater resting glycogen than thin ponies. Thin ponies (BCS of 3 and 4) having 10.73 mg glycogen/g protein, moderate ponies (BCS 5 and 6) having 13.16, and fat (BCS 7 and 8) having 14.22, and these resting values increased to 13.85, 17.93, and 18.33 mg glycogen/g protein, respectively, following fat supplementation (Jones et al., 1992). Less ($P < 0.05$) resting glycogen concentration present in thin horses compared to horses with moderate body condition is important because performance horses are often kept thinner than idle horses. As mentioned previously, differences in fiber type prevalence exist between individual horses and between breeds, which could explain variation in glycogen concentration that exists among studies.

In many disciplines, horses compete in several bouts of exercise within a single day or on consecutive days, and the length of time between events may not be adequate for sufficient repletion of glycogen stores (Snow and Harris, 1991). The effect of muscle glycogen depletion on subsequent performance has been slightly unclear and appears to be dependent upon the degree of depletion. In a study conducted by Davie and colleagues (1996), run time to fatigue of six Thoroughbred geldings during high intensity exercise was not affected by a depletion of muscle glycogen by 22 %. Lacombe and others (1999) found that exercise which decreased muscle glycogen stores by 55 % compared to pre-exercise concentration was associated with a marked decrease in anaerobic capacity during subsequent high intensity exercise. Other confounding factors, such as dehydration and muscular pain, may contribute to the observed decrease in performance.

Oldham and colleagues (1990) found that fat-supplemented horses had greater glycogen mobilization during exercise and had decreased run times during 600 m gallops compared to control fed horses. In a similar study with fat-supplemented miniature horses of varying BCS, researchers found that muscle glycogen utilized during exercise increased in all ponies, regardless of BCS (Jones et al., 1992). In the same study, the increased utilization of glycogen in fat-supplemented ponies was accompanied by a corresponding increase in peak post-exercise plasma lactate.

As mentioned previously, glycogen depletion in muscle is dependent upon fiber type within the muscle, with type I fibers being recruited first, type IIA recruited second, and IIB last. Standardbred Trotters were found to show glycogen depletion in 82 % of fibers, and type I fibers had the greatest depletion and least in IIB (Valberg, 1986). In the same study all horses displayed depletion in all type I and IIA fibers, but individual

variation was present in IIB fibers. Valberg also evaluated depletion patterns in horses at varying exercise intensities in this study. Horses that trotted at slow speeds of 6 m/s displayed glycogen depletion only in highly oxidative type I fibers and minimally in IIA fibers, and IIA fibers were increasingly recruited at increasing speeds. Horses did not display major recruitment of IIB fiber until trotting speeds of 12.5 m/s or greater were reached. Horses displayed the greatest depletion in type I fibers followed by IIA fibers, with IIB fibers displaying the least depletion. Contradictory studies have reported that maximal exercise resulted in depletion of glycogen in type IIA and IIB fibers, but not in type I fibers. Thoroughbreds, for example, were found not to have appreciable depletion of type I fibers, but that types IIA and IIB did when Periodic Acid Schiff (PAS) stain intensity was used (Hodgson et al., 1984). The difference in utilization of glycogen and recruitment of fibers likely can be explained by differences in the muscle force generation required for trotting and galloping, although another study conducted with Standardbred Trotters also displayed preferential recruitment of type I, then type IIA, and finally type IIB fibers (Lindholm and Peihl, 1974), and this is generally accepted as the normal pattern of recruitment.

Introducing an incline to exercise tests can result in increased muscle glycogen depletion at slower speeds. Valberg (1986) found that in a treadmill exercise test with a 6.25$^{o}$ grade, along with increased velocity every 2 min, found that all fiber types in the gluteus medias were recruited and displayed depletion, but that none of the fibers were completely depleted. Depletions of muscle glycogen during this study found that the recruitment pattern was the same as the normal pattern mentioned previously, but none of the fibers were recruited to the full extent. This could be the result of the combination of

the effects of uphill exercise and continually changing demands of muscle fibers as the speed of exercise also continually changed.

Lindholm and colleagues (1974) found that equine muscle glycogen depletion was dependent upon exercise intensity and length of exercise. Horses exercised at a slow trot for 4 h had a mean glycogen depletion rate of 21 mmol/kg/h for the initial 3 h of exercise, but the rate of depletion declined dramatically to 8 mmol/kg/h during the final h of exercise when horses were no longer able to maintain pace despite the availability of glycogen in all fiber types. Slow trotting horses had post-exercise muscle glycogen concentrations of 48 mmol/kg compared to 119 before exercise. Horses trotting at a fast pace for 1 h had a muscle glycogen concentration of 70 mmol/kg after 60 min of exercise and 43 after 30 min of exercise, compared to 103 before exercise. Horses completing the fast trot exercise test metabolized glycogen 3 times faster than horses competing the slow trot exercise test.

Hodgson and others (1983) investigated glycogen depletion patterns in horses of unspecified breed competing in endurance rides. Horses competing in 40 and 160 km rides both displayed muscle glycogen depletion following their rides. As expected, horses competing in the 160 km had the greatest depletion. These horses displayed complete glycogen depletion in 70 % of type I fibers with PAS staining. Glycogen repletion in horses participating in this study was found to occur in the reverse order of recruitment, with type IIB fibers being replenished first, followed by IIA and then I. Repletion of IIB fibers took 24 or more h following exercise, and glycogen of types I and IIA was not replenished until 48 h post-exercise. Horses that competed in the 160 km ride displayed no repletion of type IIB fibers with PAS staining 18 to 20 h after the endurance ride. The

investigators recommended restricting exercise following endurance exercise in order to maximize repletion of muscle glycogen.

Thoroughbred horses completing 1,000 and 1,600 m gallops were found to have 19 % to 25 % decrease in glycogen concentrations compared to resting values and returned to resting concentrations within 72 h (Snow and Guy, 1991). Kitaoka and others (2014) sought to determine the effects of 1 and 2 min of strenuous exercise at 120 % of maximal $VO_2$ on a treadmill with a $6^o$ incline on glycogen metabolism. Muscle glycogen decreased to 42 % of initial concentration after 1 min and 41 % of initial concentration after 2 min of exercise. A study conducted by Davie and others (1999) determined the effect of submaximal treadmill exercise on muscle glycogen depletion in Thoroughbreds. Some horses underwent a less intense protocol designed to deplete primarily type I fibers, while others underwent a short, more intense exercise protocol meant to deplete type II fibers. All horses underwent another exercise bout 5 h following the initial protocol in which they were exercised at 60 % of their maximal $VO_2$. Initial protocol was not a determinant in the degree of final muscle glycogen depletion, as glycogen depletions for the protocols were not different. Muscle glycogen was depleted by 21 % and 29 % for the less intense and more intense protocols, respectively.

In a study by Hyyppä and colleagues (1997), Standardbred Trotters completed 2 separate exercise protocols. Horses were first trotted distances of 3, 000, 3,000, and 2,000 m, and this was termed Trial A. Three days later in Trial B, horses were trotted 2,100, 2,100, and 1,600 m. In each protocol, horses were allowed a 1 h rest period between each exercise bout. A basal muscle glycogen concentration of 473 ± 45 mmol/ kg dry weight in horses prior to exercise, which was depleted to 329 ± 79 following Trial A. Only a

small amount of resynthesis had occurred 24 h after exercise, but muscle glycogen concentration had returned to basal values of 472 ± 128 mmol/kg dry weight 72 h following Trial A. Muscle glycogen concentration was depleted to 347 ± 39 mmol/ kg dry weight immediately after exercise in Trial B. Contrary to the results from Trial A, muscle glycogen was not returned to basal concentrations following Trial B. In fact, 72 h following Trial B muscle glycogen concentration was found to be 279 ± 52 mmol/kg dry weight. Additionally, further glycogen depletion was apparent 4 h following exercise in both protocols. Glucose concentrations for horses in each exercise protocol were greater or equal to pre-exercise concentrations, so this lack of glycogen repletion cannot be attributed to hypoglycemia. Research involving multiple exercise protocols occurring over multiple days is of particular interest, not only because it simulates the realistic exercise undertaken by performance horses, but because of the rate of muscle glycogen repletion apparent in horses compared to humans. Human exercise trials have found that glycogen repletion rate was diminished slightly in limbs that underwent mild exercise during a recovery period from glycogen-depleting exercise (Bonen et al., 1985). These results suggest that repeated bouts of exercise can negatively impact glycogen repletion, which is supported by the fact that glycogen repletion was impaired by repeated exercise in horses following Trial B (Hyyppä et al., 1997).

The potential for impaired athletic performance in consecutive exercise bouts has led to further research in relation to potential negative effects of fatigue during subsequent exercise. Marlin and Nankervis (2002) explained that “fatigue” is a complex issue that is dependent upon the type of exercise being performed, but that, in general, the term refers to a horse’s inability to keep pace with a particular exercise intensity. Fatigue

may be the result of multiple factors including depletion of muscle or liver glycogen, muscle acidosis, or muscle injury.

## 1.7 Respiratory Response to Exercise

Both oxygen consumption ($VO_2$) and carbon dioxide expelled ($VCO_2$) increase as horses transition from rest to exercise. Oxygen consumption and submaximal exercise intensity seem to have a linear relationship such that as one increases, so does the other (Marlin and Nankervis, 2002). Similarly to glycolytic response, respiratory response to exercise can be influenced with training. Trained horses typically have greater maximal $VO_2$ than their untrained counterparts (Snow and Mackenzie, 1977).

Six previously trained Thoroughbred geldings were allowed to become unfit and were then used in a treadmill exercise test at a trot, canter, and gallop in order to determine the relationship between $VO_2$ and speed, heart rate (HR), and other cardiovascular parameters (Evans and Rose, 1988). Horses in this study were found to have a resting mean $VO_2$ of 3 ml $\bullet$ $kg^{-1}$ $\bullet$ $min^{-1}$. Maximal $VO_2$, which occurred at a speed of 8.0 m/s, increased from resting values by nearly 40 times at 129 ml $\bullet$ $kg^{-1}$ $\bullet$ $min^{-1}$. At maximal $VO_2$, horses displayed a HR of 222 ± 7 beats/min when the treadmill was at a slope of $10^{o}$.

Rose and colleagues (1990) exercised untrained Thoroughbreds on a treadmill to determine the respiratory effects of various exercise tests. Three protocols utilized a 10 % slope, the first of which involved increasing the speed of exercise by 1 to 2 m/sec every 1 min from 4 m/sec to a maximum 12 m/sec. The second test included exercising horses at 12 m/sec until the horses reached fatigue. The third exercise test was composed of

exercising for 3 to 4 min at speeds ranging from 6 to 12 m/sec with rests between each exercise bout. The fourth and fifth exercise tests involved exercising horses at 24 % incline. A plateau in $VO_2$ occurred in all exercise protocols except those in which the horse was exercised on a 24 % slope. Horses exercised at slow speeds on a 24 % slope displayed the greatest $VO_2$, though there was no plateau present. A steady state for $VO_2$ and $VCO_2$ was present during all protocols when horses were exercised at a constant speed for 2 min or more.

Segal and Brooks (1979) conducted a study in which 11 human males were subjected to exercise on a bicycle at both moderate and heavy intensities. Decreased blood glucose, blood lactate, minute ventilation (VE), and $VCO_2$ were observed in glycogen-depleted subjects at rest. These variables were decreased further in response to heavy exercise, with the exception of blood glucose. Another human study by Heigenhauser and others (1983) found that a reduction in muscle glycogen to 17.1 μmol/g following exercise from 59.1 before exercise was associated with a 14% maximum power output but not with a change in maximum $O_2$ intake. It was determined that for any amount of power output, $VO_2$, HR, and VE were greater, and that $VCO_2$ was similar. Respiratory exchange ratio refers to ratio of carbon dioxide produced during metabolism and the oxygen that was used during metabolism (Marlin and Nankervis, 2002). Glycogen depletion was associated with a decreased respiratory exchange ratio compared to control values (Heigenhauser et al., 1983).

Respiratory frequency ($R_f$), the number of breaths taken per minute, and tidal volume (VT), the volume of air inhaled or exhaled during a single breath, are among other parameters commonly evaluated in exercise physiology. A study of exercising

humans sought to evaluate the relationship between $R_f$ and $VO_2$ during exercise and found that although several $R_f$ values were observed during exercise, the total oxygen uptake was unaffected by altered $R_f$ (Kennard and Martin, 1984).

Interestingly, there is a phenomenon known as locomotor-respiratory coupling in quadrupeds which states that there is an obligatory synchronization of locomotor and respiratory cycles at a ratio 1 stride to 1 breath (Giuliodori et al., 2009). In horses, locomotor- respiratory coupling occurs during fast canters and during gallops at all times with the exception of when a horse swallows, during acceleration, and sometimes when changing leads (Marlin and Nankervis, 2002). Due to this coupling, horses must alter tidal volume during a canter and gallop in order to maintain an adequate oxygen supply (Giuliodori et al., 2009). A study by Butler and colleagues (1993) used horses exercised at varying speeds, yet maintained a constant metabolic demand by altering treadmill incline. It was determined that horses are able to meet altered metabolic demands which are not directly related to speed (and therefore stride) by altering VT in response to increased intensity of exercise independently of stride length. The horses were not, however, able to alter $R_f$ independently of stride length. Both stride length and stride frequency increase with increasing speed (Shuback et al., 1999), and so it is reasonable to conclude that $R_f$ would also increase with speed.

## 1.8 Nutritional Intervention

Bergström and colleagues (1967) demonstrated that muscle glycogen replenishment following exercise could be altered with diet, and that humans consuming starch following exercise displayed increased glycogen resynthesis. Since this time, much

research has been conducted regarding dietary manipulation of carbohydrates in order to maximize muscle glycogen resynthesis.

Ivy and others (1988) conducted a study during which 12 male cyclists exercised for 70 min continuously on a cycle ergometer at 68 % maximal $VO_2$ for 8 min intervals interrupted by two-min intervals at 88 % maximal $VO_2$ and concluded with 10 min at 68 % maximal $VO_2$. Subjects then immediately or 2 h after exercise ingested a 25 % glucose-polymer solution at a rate of 2 g/kg BW. Muscle samples from the vastus lateralis were taken 0, 2, and 4 h postexercise. Subjects that received carbohydrate supplementation immediately after exercise replenished glycogen at a rate of 7.7 μmol • g wet weight$^{-1}$ • h$^{-1}$, and those supplemented 2 h postexercise replenished glycogen at a rate of 2.5 μmol• g wet weight$^{-1}$ • h$^{-1}$ during the first 2 h after exercise. Glycogen repletion for the immediately supplemented group slowed 4.3 μmol• g wet weight$^{-1}$ • h$^{-1}$ during the second 2 h after exercise. Repletion increased to 4.3 μmol• g wet weight$^{-1}$ • h$^{-1}$for the 2 h postexercise supplemented group, which is still 45 % slower than repletion during the first 2 h postexercise for the immediately supplemented group. These results suggest that immediate glucose ingestion effectively improves glycogen repletion in humans compared to delayed ingestion.

In a second study, Ivy and colleagues (1988) sought to determine whether amount of carbohydrate ingested after exercise affected glycogen repletion. Subjects exercised on a cycle ergometer for 2 h on 3 occasions in order to deplete muscle glycogen, and then ingested either 0, 1.5, or 3.0 g glucose/kg BW from a 50 % glucose solution either immediately or 2 h postexercise. Rates of muscle glycogen replenishment were not different for 1.5 and 3 g glucose/kg BW groups during the first hour of recovery, with

repletion rates of 5.2 ± 0.9 and 5.8 ± 0.7 μmol/g wet weight/h, respectively. Repletion rates were not different for the 2 groups during the second hour of recovery (4.0 ± 0.9 and 4.5 ± 0.6 μmol/g wet weight/h for 1.5 and 3 g glucose/BW groups, respectively). Carbohydrate, protein, and carbohydrate-protein supplementation were compared in a study by Zawadzki and others (1992) that studied the effects on muscle glycogen repletion. Immediately and 2 h following glycogen-depleting exercise, human subjects ingested either 112.0 g carbohydrate (21 % wt/volume dextrose-maltodextrin mixture), 40.7 g protein (7.6 % milk and whey protein isolate mixture), or 112.0 g carbohydrate and 40.7 g protein. The researchers discovered that muscle glycogen repletion occurred most quickly with the carbohydrate-protein diet at a rate of 35.5 ± 3.3 μmol/g protein/h, followed by the carbohydrate only diet at a rate of 25.6 ± 2.3, and finally by the protein only diet at a rate of 7.6 ± 1.4. These results suggest that muscle glycogen can be enhanced by inclusion of protein along with carbohydrate supplementation following exercise and have been confirmed by later studies (Van Loon et al., 2000; Jentjens and Jeukendrup, 2003).

Coingestion of both fat and protein in a carbohydrate diet following glycogen-depleting exercise was not found to further increase the rate of glycogen repletion and resulted in decreased total plasma glucose compared to an isocaloric carbohydrate diet (Burke et al., 1995; Burke et al., 2016). Rats adapted to an increased fat diet had an increased run time in an exercise test, despite having decreased basal glycogen concentrations (Conlee et al., 1990). This increase in endurance was likely due to an acclimatization to utilization of triglycerides as an energy substrate and a subsequent glycogen sparing effect, which could have been the result of increased presence of

mitochondrial enzymes responsible for oxidative phosphorylation of metabolites derived from fatty acids (Korzeniewski and Zoladz, 2003).

The success of dietary manipulation of muscle glycogen resynthesis apparent in humans and other species has led to a plethora of research concerning whether this is also an effective method in horses. Similarly to humans, the length of time needed for return of muscle glycogen concentration to basal concentration is influenced not only by the degree of depletion, but also by the timing and amount of carbohydrates delivered following exercise (Lacombe et al., 2003). Developing nutritional strategies that minimize the length of time of recovery after exercise may be even more crucial for horses due to the increased length of time needed to replenish muscle glycogen relative to humans (Lacombe et al., 2003).

Davie and others (1994) administered either a glucose polymer at 1.5 g of glucose polymer/kg BW or an equivalent volume of water either 30 min following exercise and another dose 3 h later or only a single dose 30 min after exercise. No effect of treatment was observed despite elevated blood glucose and insulin concentrations following glucose administration, suggesting that blood glucose concentrations may not have been sufficiently different to affect glycogen resynthesis or that glucose uptake from the bloodstream or absorption from the gastrointestinal tract may be a limiting factor in glycogen resynthesis. Pösö and colleagues (1999) found that administration of propionate, a gluconeogenic precursor, via a nasogastric tube did not affect the rate of glycogen replenishment in treadmill exercised horses.

Contradictory evidence exists suggesting that muscle glycogen resynthesis can be affected by carbohydrate supplementation after exercise. A study investigating the effects

of low, moderate, and high-soluble carbohydrate diets on glycogen repletion rates found that horses consuming moderate and high soluble carbohydrate diets had increased rates of glycogen replenishment compared to the low soluble carbohydrate diet (Snow et al., 1987). These diets, however, were not isocaloric. Moderate and high soluble carbohydrate diets provided 32.0 and 34.0 Mcal/d respectively, while the low soluble carbohydrate diet provided 20.7 Mcal/d. A similar study by Vonderhoe and colleagues (2013) found that horses fed increased starch diets (997.6 g starch/d) had increased rates of glycogen repletion compared to horses fed less starch (553.7 g starch/d). Additionally, horses fed an increased starch diet had increased muscle glycogen compared to horses fed less starch, despite horses fed both diets having reached basal muscle glycogen concentrations within 48 h. In an effort to meet increased energy demands due to exercise and also minimize the potential health issues that arise from increased starch intake, high fat diets have been developed and are becoming more common (Warren, 2004). Inclusion of dietary fat supplementation had no effect on resting muscle glycogen depletion in a study conducted by Eaton and colleagues (1995). Other studies have found that fat supplementation to horses prior to exercise can increase glycogen concentration in resting muscle (Oldham et al., 1990; Eaton et al., 1995).

## 1.9 Conclusion

Metabolism of glycogen and its subsequent replenishment following exercise are objects of study and importance to the equine industry due to the intense exercise that performance horses undergo within a short interval of time. Research in this area has focused on the effect of carbohydrate supplementation and timing of supplementation

relative to exercise in order to minimize the time needed for replenishment of glycogen following intense exercise. Attempts to meet this need have yielded inconclusive results. Breed differences in muscle fiber type resulting in differences in glycogen metabolism and inconsistent exercise protocols have made it difficult to compare results of glycogen depleting exercise among studies.

The objectives of the current study were to utilize a ridden field exercise test in order to determine the effect of hay quality on:

1. Voluntary hay intake.
2. Metabolic parameters such as serum glucose, serum insulin, muscle glycogen, whole blood lactate, heart rate, and respiratory factors before and after exercise.

# CHAPTER II

# MATERIALS AND METHODS

## 2.1 Animal Management

Stock-type mares (n= 6) and geldings (n = 6) were used in a completely randomized study lasting 4 wk. Horses ranged from 4 to 20 yr (mean age of 13.4 ± 4.62 yr) with an average BW of 494 ± 38.0 kg, and were selected based on temperament, health, and soundness. Unshrunk body weight of horses was obtained at the beginning of the trial and every 7 d until the end of the trial. Horses were housed individually at the Mississippi State University Horse Unit in stalls, and horses were managed in accordance with guidelines established by the Institutional Animal Care and Use Committee.

During the first week of the study, horses were allowed ad libitum access to one of two Annual Ryegrass (*Lolium multiflorum*) hays of differing qualities and did not receive any concentrate. Beginning the second week of the study, horses received 1.5% BW of their assigned hay and were acclimated to the concentrate portion of their diet. Horses received both concentrate and hay during the third week before performing a ridden standardized exercise test (**RSET**) during the fourth week.

## 2.2 Dietary Treatment

Two Annual Ryegrass hays were utilized: either high quality hay (61.3 % NDF; 32.9 % ADF) or low quality hay (70.7 % NDF; 43.7 % ADF). Horses received a commercially available concentrate (Nutrena® SafeChoice Original; 33.1 % NDF; 15.8

% ADF) in addition to hay resulting in 3 possible diets: high quality hay and concentrate balanced to meet energy requirements assuming moderate activity (HQ); low quality hay and concentrate balanced to meet energy requirements assuming moderate activity (LQ); and low quality hay receiving the same amount of concentrate as the HQ diet assuming moderate activity (LQ-). Daily hay and concentrate rations were divided into 2 equal meals fed at 0700 and 1600 h. Hay and concentrate were offered to each horse in an individual feeder. Orts were collected and weighed daily. Horses had ad libitum access to water throughout the trial. Nutrient analysis of Nutrena® SafeChoice Original, high quality, and low quality hay is presented in Table 1.

During the second week of the study, horses were acclimated to the concentrate portion of their assigned diets during which they received 25 % of their full concentrate for the first 2 d of the acclimation period, 50 % on d 3 and 4, and 75 % on d 5, 6, and 7, and fed 1.5 % BW of hay from day 8 until the end of their trial.

### 2.3 Exercise Protocol

Prior to the beginning of the trial, horses were exercised lightly as pleasure riding horses or as a part of the Mississippi State University riding program. During the trial, horses were lightly exercised to acclimate them to the use of a Cosmed K4b2 respiratory mask. The RSET was conducted on a track at the Mississippi Horse Park in Starkville, MS and was based on previous protocols which utilized incremental gallop sprints (Lacombe et al., 1999; Pagan and Harris, 1999; Barneveld, 1995). Each horse performed the RSET once. During the first 5 min of the RSET, horses were walked (1.5 m/s), then trotted for 10 min (4.5 m/s), cantered for 8 min (5.5 m/s), and walked for 2 min (1.5 m/s). Horses then performed four 2 min gallop sprints (6.5, 7.5, 8.5, and 9.5 m/s). Between

each sprint, horses were walked for 2 min (1.5 m/s). Following the final gallop, horses were walked for 5 min (1.5 m/s) before the conclusion of the RSET. Body temperature was recorded immediately before and after RSET. To allow for time to conduct all 12 RSET, beginning time for horses were staggered such that three (1/diet) started the trial first, then six (2/diet) started the following week, and finally three horses (1/diet) started the following week. Following the RSET and immediate muscle biopsy and venous blood collection procedures, horses were hand walked to allow them to cool down, were hosed and sponged off, and were offered ad libitum access to water after recovery from RSET. Environmental temperature and relative humidity were recorded at the beginning of each RSET. Environmental temperature ranged from 22.8 to 31.7 $^{o}$C, and relative humidity ranged from 69 to 94 %.

Nine of the 12 horses were equipped with a Cosmed K4b2 respiratory mask in order to acquire values for $R_f$, VT, $VO_2$, $VCO_2$, and HR. Horses were acclimated to the mask at least 2 wk prior to the RSET. The respiratory mask was calibrated before each use.

## 2.4 Sample Collection

### 2.4.1 Muscle

Biceps femoris muscle biopsies were collected immediately before the RSET, immediately following the conclusion of the RSET, and 24, 48, and 72 h post-RSET from alternating sides of the horse. Muscle biopsies were collected at a consistent depth of 4 to 5 cm. Horses were restrained in stocks during the procedure, and a humane lip twitch was used in the event that either horse or handler safety were in jeopardy.

Immediately before the first muscle biopsy procedure, an area superior to the biceps femoris that was approximately 5.08 cm wide and 7.62 cm long was clipped using a #40 clipper blade. This procedure was conducted on both sides of the horse. Before each biopsy was collected, the clipped area was aseptically disinfected using betadine scrub, chlorohexidine, and 70 % isopropyl alcohol. The site was allowed to sit for approximately 1 min to allow for complete disinfection by alcohol spray. The area immediately proximal to the site of the muscle biopsy was locally anesthetized with approximately 1.5 mL lidocaine administered subcutaneously with a 20G x 2.54 cm needle 2 min before the procedure was continued.

Following the administration of lidocaine, a stab incision was made using a 14G needle, to create a small puncture site for the biopsy needle. Muscle biopsies were collected with a mechanized modified Bergstrum biopsy needle. The biopsy needle was inserted into the initial puncture, and the needle was inserted until the muscle fascia was penetrated. Once the cutting window of the biopsy needle was pushed past the fascia, the inner cannula was pulled back to expose the cutting window. The inner cannula was held open for approximately 30 sec to allow the muscle to settle across the cutting window. The inner cannula was then shut to cut the muscle sample, and the biopsy needle was removed from the muscle. The sample was removed from the cutting window with forceps and placed in a cryotube vial and immediately placed in liquid nitrogen and then stored at -80°C until analysis.

Following the biopsy procedure, the site was cleaned once more with betadine and isopropyl alcohol. Pressure was applied to the area until the area no longer bled. To prevent infection, an antiseptic spray (Blu-Kote, H.W. Naylor Co., Inc., Morris, NY) was

applied after the area dried, followed by an aerosol bandage (AluShield, Neogen, Lexington, KY). Horses were monitored for swelling, infection, and lameness following the biopsy, but no problems were observed.

### 2.4.2 Blood

Blood was collected by jugular venipuncture into sterile vacutainers containing no anticoagulant at the time of each biopsy. Approximately 1 mL was removed immediately for whole blood lactate analysis. Tubes were inverted and placed horizontally during transportation. Blood samples were allowed to clot and centrifuged within 45 min of collection at 2750 ・g for 15 min. Serum was then removed and sub-samples stored in 1.5 mL microcentrifuge tubes at -20 °C until analysis.

### 2.4.3 Hay and Concentrate

Representative core samples were taken from random bales of HQ and LQ hay to allow for formulation of diets. Throughout the study, grab samples were taken from each bale opened. Additionally, grab samples were taken from each bag of concentrate that was used. Orts were collected and weighed each day at 0700 h, and samples representative of 10% of the total orts collected were taken. Samples were compiled and stored at -20 °C until they were ground through a 2 mm screen using a Thomas Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ). They were then stored at -20 °C until analysis.

## 2.5 Sample Analysis

### 2.5.1 Glycogen

Muscle samples were analyzed using a commercial glycogen colorimetic/fluorimetric ELISA kit (Biovision Inc., Milipitas, CA). Frozen muscle samples were weighed and an average sub-sample of 13.5 mg was removed and used for analysis. Individual sample wet weights were recorded to account for individual variation in muscle glycogen concentration during statistical analysis. Muscle sub-samples were homogenized in 200 µL of distilled water. Samples were boiled for 10 min to ensure enzymes were inactivated. Samples were then centrifuged at 18,000 · g at 4°C for 10 min. The supernatant was then removed and stored at -20 °C until analysis. Five microliters of sample were pipetted onto a 96-well plate in duplicate. Hydrolysis buffer was added to each well to bring the total volume to 50 µL. The hydrolysis enzyme mix was added to each well at a volume of 2 µL per well. The plate was then incubated at room temperature for 30 min before 50 µL of the reaction mix containing development buffer, development enzyme mix, and OxiRed probe was added to each well. The reaction mix was formulated such that 50 µL was prepared for each well. Forty-six microliters of development buffer, 2 µL of development enzyme mix, and 2 µL of OxiRed probe were mixed per well. After the reaction mix was added, the plate was incubated in a dark room for 30 min. Absorbance was measured using a well reader (Molecular Devices SPECTRAmax Plus) at 570 nm.

Glycogen concentration was determined using a glycogen standard. This was prepared by diluting the glycogen standard to 0.2 mg/mL by adding 10 µL of the standard to 90 µL of distilled water and mixing the combination well. The standard was added to a

series of wells at a volume of 0, 2, 4, 6, 8, and 10 µL in duplicate. The volume was adjusted to 50 µL per well with hydrolysis buffer to generate 0, 0.4, 0.8, 1.2, 1.6, and 2.0 µg/well of glycogen standard. The remainder of the glycogen standard procedure was identical to the procedure detailed previously.

The kit functioned by generating a product from the hydrolysis of glycogen to glucose by glucoamylase, which was then oxidized to produce a second product that reacted with the OxiRed probe. The kit was able to detect glycogen from 0.0004 to 2.0 mg/mL. The 6 glycogen standard wells were then used to generate a standard curve. The glycogen concentration (µg/mg of wet weight) was calculated from the standard curve. Three 96-well plates were utilized to run the samples.

### 2.5.2 Lactate

A small amount of whole blood was immediately analyzed upon collection for lactate using a Lactate Plus handheld lactate analyzer (Nova Biomedical, Waltham, MA). Concentration was expressed in mmol/L. The Lactate Plus has been validated to reliably measure lactate concentrations ranging from 0 to 8 mmol/L (Hauss et al., 2014).

### 2.5.3 Glucose

Serum samples were analyzed using a commercially available glucose colorimetric assay kit (Caymen Chemical, Ann Arbor, MI). Ten milliliters of sodium phosphate assay buffer was diluted with 40 mL of distilled water. The assay was run using a 96-well plate, and 85 µL of diluted assay buffer was added to each well. Each sample was pipetted into a well in duplicate at a volume of 15 µL. The enzyme mixture was added to each well at a volume of 100 µL, and the plate was incubated for 10 min.

Absorbance was then measured using a well reader (Molecular Devices SPECTRAmax Plus) at 510 nm. Samples were analyzed using three 96-well plates.

Glucose standards were prepared by 0, 20, 40, 80, 100, 120, 160, and 200 µL into 1.5 mL microcentrifuge tubes. The total volume was brought to 200 µL with the diluted assay buffer to bring the glucose concentration to 0, 10, 20, 40, 50, 60, 80, and 100 mg/dL respectively. Glucose standards were analyzed identically to the procedure detailed previously for serum samples.

This assay functioned by utilizing glucose oxidase which oxidized glucose to δ-gluconolactone. The reduced form of glucose was then regenerated to its oxidized form by molecular oxygen to form hydrogen peroxide. Horseradish peroxidase then acted as a catalyst for a reaction between 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine. This reaction generated a pink dye, the absorbance of which was detected by the plate reader at 510 nm.

### 2.5.4 Insulin

Serum samples were analyzed using a commercially available equine insulin ELISA kit (Mercodia, Uppsala, Sweden). Enzyme conjugate 1X solution was prepared by diluting 1.3 mL of enzyme conjugate 11X solution with 13 mL of enzyme conjugate buffer. Twenty-five microliters of each sample were pipetted into a well in duplicate. The enzyme conjugate 1X solution was then added to each well at a volume of 100 µL. The plate was incubated for 2 h at room temperature on a plate shaker (250 rpm). Following the incubation period, the reaction volume was discarded by inverting the plate over a sink. Wash buffer 1X solution was prepared by diluting 50 mL of wash buffer 21X with 1,000 mL distilled water. Wash buffer 1X solution was added to each well at a volume of

350 µL. The wash buffer 1X solution was then discarded, and this wash procedure was repeated 5 times. Substrate 3,3'-5,5'-tetramethylbenzidine (TMB) was added to each well at a volume of 200 µL, and the plate was incubated for 15 min at room temperature. Following the incubation, 50 µL of stop solution was added to each well, and the plate was placed on a plate shaker for approximately 5 sec to assure sufficient mixing. The absorbance then was read at 450 nm on a well reader (Molecular Devices SPECTRAmax Plus). Insulin standards supplied in the kit were used to generate an insulin standard curve. Standard concentrations were 0.02, 0.05, 0.15, 0.5, and 1.5 µg/L. Twenty-five microliter of standards were added to standard wells in duplicate. The procedure for standard wells was then identical to the procedure for sample wells described previously.

The kit was based on direct sandwich technique. Peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microtitration wells reacted with insulin during the incubation period. During the washing procedure, unbound enzyme-labelled antibody was removed. The bound conjugate was detected by a reaction with substrate TMB, and this reaction was halted by the addition of the stop solution containing 0.5 M $H_4SO_4$. The absorbance of the resulting product was read spectrophotometrically.

### 2.5.5 Hay and Concentrate

Orts, concentrate, and hay samples were analyzed for % DM, CP, ash, and ether extract (AOAC, 1990) and NDF and ADF (Van Soest et al., 1991). Nutrient compositions for Nutrena® SafeChoice Original, HQ hay, and LQ hay are available in Table 4.3.

### 2.5.6 Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (V. 9.4, SAS Inst. Inc., Cary, NC) as a completely randomized design with repeated measures. The model included diet, sex, time, diet x sex, and diet x time as fixed effects. Horse was included as a random effect, and horse within diet was the subject of repeated measures. Differences were considered significant at a $P \leq 0.05$. Tendencies were considered at $0.1 \geq P > 0.05$.

# CHAPTER III

# RESULTS

## 3.1 Intake

Digestible energy (DE) obtained from hay was calculated using the equation DE (Mcal/kg) = 4.22 – 0.11 x (%ADF) + 0.0332 x (%CP) + 0.00112 x (% $ADF^2$), obtained from the NRC (2007). Digestible energy obtained from concentrate was determined using the equation DE (Mcal/kg) = 4.07 – 0.055 x (%ADF), obtained from the NRC (2007). Hay was fed at a rate of 1.5 % BW per d and remaining calories were met by feeding a concentrate. Body weight of horses was obtained (Table 2) and was unchanged throughout the trial, and thus amount of hay and concentrate fed did not change for each horse throughout the trial. Hay intake (Table 3) was affected by time during the 7 d ad libitum period ($P < 0.0001$). Hay intake increased on d 2 ($P = 0.0001$) and d 3 ($P = 0.0029$) compared to d 1. Hay intake was further increased on d 4 through d 7 compared to day one ($P = 0.0013$), but was unchanged after d 4.

Daily nutrient intake for all diets is presented in Table 4. Dry matter intake was not different among diets ($P = 0.3407$). There was an effect of diet on NDF ($P < 0.0001$) and ADF intake ($P < 0.0001$). Horses fed LQ- had the greatest NDF intake ($P < 0.0001$), followed by horses fed LQ ($P < 0.0001$) ,and horses fed HQ had the least ($P < 0.0001$). Horses fed LQ- had greater ADF intake than horses fed HQ ($P < 0.0001$) and horses fed LQ ($P < 0.0001$). Horses fed HQ and LQ did not have different ADF intake (0.2761).

Diet did not affect ash intake (P = 0.5686) or ether extract intake (P = 0.5642). Crude protein was affected by diet (P < 0.0001) and was greatest in horses fed HQ, followed by horses fed LQ, and was the least in horses fed LQ-.

### 3.2 Skeletal Muscle Glycogen

There were both a diet x sex (P = 0.0243; Table 5) and diet x time (P = 0.0445; Table 6). Muscle glycogen of geldings was not affected by diet. Mares consuming HQ had the least muscle glycogen and those fed LQ- had the greatest (P = 0.0018), with mares fed LQ having intermediate muscle glycogen (P = 0.4254).

Only horses consuming the HQ diet had depleted glycogen following the RSET (P = 0.0238). These horses had 67 % of resting muscle glycogen concentration immediately following the RSET, and glycogen for these horses was replenished to 106 % of resting concentration within 24 h (P = 0.0051). Muscle glycogen concentrations of horses consuming LQ and LQ- were not affected by RSET.

### 3.3 Serum Insulin

Means of serum insulin concentration for diets and sample times are presented in Table 7. There was no effect of time on serum insulin concentration (P = 0.8136). Mares and geldings did not display different insulin responses (P = 0.7264). Diet did not affect serum insulin concentration (P = 0.7264).

### 3.4 Serum Glucose

Means for serum glucose for diets are presented in Table 7. A diet x sex interaction was observed (P = 0.0001), and results can be found in Table 8. Geldings consuming LQ had greater serum glucose than did mares consuming LQ hay. Mares

consuming HQ hay had greater serum glucose than did mares or geldings consuming either of the LQ diets. An effect of time ($P = 0.0009$) on serum glucose concentration was observed, and these means are presented in Table 9. Serum glucose peaked immediately after exercise and returned to pre-exercise values within 24 h. Blood glucose concentrations were decreased before exercise vs. immediately after exercise ($P < 0.0001$), increased immediately after exercise vs. 24 h post exercise ($P = 0.0009$), increased immediately after exercise vs. 48 h post exercise ($P = 0.0071$ ), and increased immediately after exercise vs. 72 h post exercise ($P = 0.0019$).

### 3.5 Whole Blood Lactate

Whole blood lactate concentration was not affected by diet ($P = 0.1525$), and mean lactate concentrations for diets are available in Table 7. Whole blood lactate was increased by the RSET ($P < 0.0001$). Lactate was increased by 120 % immediately after exercise compared to pre-exercise concentration ($P < 0.0001$), and the results are presented in Table 9. Lactate returned to pre-exercise values by 24 h post-exercise. There was tendency for mares to have greater whole blood lactate concentration than geldings ($P = 0.0715$). Overall means for mares and geldings are presented in Table 10.

### 3.6 Body Temperature

Means of body temperature before and after the RSET are presented in Table 11. An effect of time was observed in regards to body temperature ($P < 0.0001$). In all treatments, body temperature was increased following the RSET. Body temperature was not affected by sex ($P = 0.7349$) or by diet ($P= 0.3902$).

### 3.7 Respiratory Frequency

Means for all diets and time points are presented in Appendix I-A and I-B. Mares and geldings were not different with respect to $R_f$ ($P = 0.8830$). A diet x gait interaction ($P = 0.0043$) was observed during RSET (Table 12). When analyzing each change of gait individually, it became apparent this interaction occurred during the canter and 4$^{th}$ gallop. During the canter, horses fed LQ had decreased $R_f$ compared to horses fed HQ ($P = 0.0002$) and LQ- ($P = 0.0037$). Horses fed LQ had decreased $R_f$ compared to horses fed both both HQ ($P = 0.0023$) and LQ- ($P = 0.0031$).

### 3.8 Tidal Volume

Means for VT for different diets at all time points are presented in Appendix II-A and II-B. No effects of diet ($P = 0.8908$), time ($P = 1.0000$), or sex ($P = 0.8188$) were observed on VT during RSET. Tidal volume was affected by gait during the RSET, and was greater during the 3$^{rd}$ gallop than during any other gait ($P = 0.0445$). Mean VT during each gait is presented in Table 13.

### 3.9 Oxygen uptake

Means for $VO_2$ for different diets at all time points are presented in Appendix III-A and III-B. No effect of diet ($P = 0.5605$) or sex ($P = 0.9787$) were observed during RSET, though there was an effect of time ($P < 0.0001$). Oxygen uptake was affected by gait during RSET ($P < 0.0001$), and increased as intensity of exercise increased initially, but plateaued after the 3$^{rd}$ walk. Mean $VO_2$ during each gait is presented in Table 13.

### 3.10 Carbon dioxide output

Means for $VCO_2$ for different diets at all time points are presented in Appendix IV-A and IV-B. No effects of diet ($P = 0.4853$) or sex ($P = 0.3777$) were observed during the RSET, though there was as effect of time ($P < 0.0001$). Carbon dioxide output was affected by gait during RSET ($P < 0.0001$), and, similarly to $VO_2$, increased as intensity of exercise increased initially, but plateaued after the 3rd walk. Mean $VCO_2$ during each gait is presented in Table 13.

### 3.11 Heart Rate

Means for HR for diets and time during the RSET are presented in Appendix V-A and V-B. Neither diet ($P = 0.3235$) nor sex ($P = 0.3148$) had an effect on HR during the RSET, though, as expected, HR was affected by time ($P < 0.0001$). Heart rate was affected by gait during RSET ($P < 0.0001$) and increased as intensity of exercise increased.

# CHAPTER IV

# DISCUSSION

## 4.1 Intake

A transition time in which horses adjusted to their respective hay diets was apparent in the current study, though this transition occurred for horses consuming all diets irrespective of hay quality. Hay consumption as percentage of BW during the ad libitum portion of the trial increased from d 1 to d 2 and remained constant on d 3. Hay consumption increased once more on d 4 and then remained constant on d 5 through d 7.

Voluntary intake of hay in the first three days was less than later in the week, but in the final 4 d of the ad libitum period was similar to previously reported values (LaCasha, 1997). Crozier and colleagues (1997) reported voluntary hay intake of tall fescue hay with 72 % NDF and 40 % ADF of 2.5 % BW following a 5 d adjustment period. The low quality hay in the current study had similar NDF and ADF percentages to the hay in the Crozier study, but peak intake was less than 2 %. Additionally, CP of the tall fescue hay utilized by Crozier and others was less than that of the high quality hay in the present study. These results suggest that NDF, ADF, and CP percentages may not be primary determinants or indicators of voluntary grass hay intake in horses.

## 4.2 Skeletal Muscle Glycogen

While geldings consuming HQ had similar mean muscle glycogen concentrations compared to the mares and geldings consuming low quality hay diets, mares had less

muscle glycogen than geldings consuming HQ and both mares and geldings consuming LQ-. Horses consuming LQ had muscle glycogen concentrations which were intermediate between mares fed HQ and mares and geldings consuming the LQ- and geldings fed HQ. Though horses consuming LQ and LQ- did not have depleted glycogen following RSET, horses consuming HQ had muscle glycogen which was depleted to 67 % of the resting concentration. These horses recovered within 24 h of RSET, and muscle glycogen was replenished to 106 % of resting concentration. Other studies have reported muscle glycogen concentration recovery within 24 h (Jose-Cullineras et al., 2006). More frequently, however, complete replenishment of glycogen stores requires 48 h or more (Hodgson et al, 1983; Snow and Harris, 1991; Lacombe et al., 2004). Unfortunately, because glycogen depletion did not occur in horses fed LQ and LQ-, repletion times cannot be compared with respect to hay quality.

That only horses consuming high quality hay had depleted glycogen following RSET suggests that these horses were more reliant on muscle glycogen as an energy source during RSET. This could have been due to greater CP concentration provided by HQ (Zawadzki et al, 1992). Contraction of both type I and II muscle fibers during exercise can lead to decreased entrance of glycolytic products into the mitochondria for oxidative phosphorylation (Zhang et al., 2014), and so the glycolytic pathway would be relied upon more heavily for energy production in exercising horses, which was observed in HQ fed horses but not in horses fed low quality hay. Crude protein was provided in excess of the daily requirement for HQ fed horses, and it is possible that excess protein was utilized for gluconeogenesis, resulting in a profusion of glucose available for glycolytic activity and replenishment of glycogen following RSET. This was not

evidenced by an increased serum glucose concentration in HQ fed horses, as serum glucose concentrations were not different among the diets. Additionally, horses consuming the low quality hay diets were likely more reliant on other sources of energy during RSET, such as free fatty acids or triglycerides. Carbohydrates in the low quality hay diets were less available than those provided by HQ. Decreased availability of glucose for LQ and LQ- fed horses likely caused them to adjust to oxidation of metabolites derived from fat in order to accommodate the lack of soluble carbohydrate. Human studies have indicated that healthy individuals are able to rely more heavily on the oxidation of fat than on metabolism of glucose when a profusion of fat is present in the diet or during a period of fasting before a meal is consumed (Andres et al., 2014; Kelley et al., 1999; Dubé et al., 2014), and so it is reasonable to think that this metabolic flexibility would also be present in healthy horses consuming low quality hay with a readily available source of body fat.

Muscle glycogen concentrations throughout the present study were much less than in previously reported studies. This may have been due to the relatively small portion of concentrate, and therefore starch, consumed by all horses, regardless of diet. Further, horses involved in this study were not conditioned prior to or during the study. Other studies have reported that resting muscle glycogen concentration can be increased with training (Marlin and Nankervis, 2002; Manabe et al., 2013; Hodgson et al., 2014). As the horses in the current study were not conditioned for RSET and were not consuming large amounts of concentrate or starch, it is possible that this resulted in decreased resting muscle glycogen concentration and utilization of muscle glycogen during RSET.

## 4.3 Serum Insulin

Serum insulin was impacted neither by time nor diet. Geldings and mares did not have differing serum insulin concentration. Mean for overall insulin concentration pooled across all time points for horses fed HQ was 9.93 mIU/L, 7.60 for horses fed LQ, and 11.54 for horses fed LQ-. All means were within range reported in previous research. Frank and others (2006) reported resting serum insulin concentration ranging from 6.4 to 21.1 mIU/L in clinically normal nonobese horses, though horses in this study were allowed ad libitum access to hay before and during blood collection. When comparing the result of the present study to the results of the study conducted by Frank and colleagues (2006), it could be suggested that hay ingestion may not affect serum insulin concentration, though further research is needed in this area.

## 4.4 Serum Glucose

Mares consuming LQ and LQ- and geldings consuming HQ had similar serum glucose concentrations overall throughout the study. Geldings consuming LQ and LQ- also had similar serum glucose concentrations throughout the study, but serum glucose was greater in these horses compared to mares consuming LQ and LQ- and geldings consuming HQ. Interestingly mares consuming HQ had greater serum glucose throughout the trial compared to all other horses. Additionally, serum glucose concentration for all treatments was greater immediately following RSET. Glucose concentration for all treatments had returned to resting values within 24 h of completing RSET. Hyyppä and others (1997) reported increased blood glucose concentration following exercise and a return to normal within 1h of exercise. The current results are in support of their findings.

Resting serum glucose concentration and glucose elevation after exercise appear to be partially determined by diet. Resting glucose concentration for all horses was slightly less than resting concentration reported by Fonseca and others (2011), who reported a resting value of 68.6 ± 5.6 mg/dL. Similarly, Phillips et al. (2017) reported a resting glucose concentration of 89.33 mg/dL in horses consuming a low starch diet, which was similar to the post-exercise glucose concentration in the present study. Horses in the study conducted by Phillips and others were consuming more concentrate and less hay than horses in the present study. Frank and others (2006) reported resting glucose concentrations ranging from 49.8 to 74.1 mg/dL in nonobese horses. Though serum glucose concentrations observed in the current study were within normal range reported for horses, consistently decreased glucose concentration throughout study suggest that horses with a greater portion of the ration contributed by hay and smaller portion contributed by concentrate, such as those in the current study, may have less circulating blood glucose compared to horses consuming greater amounts of concentrate, and this is supported by studies which have found horses consuming high fiber diets have decreased peak glucose and areas under the curve compared to horses consuming greater amounts of concentrate (Stull and Rodiek, 1988; Williams et al., 2001).

### 4.5 Whole Blood Lactate

Whole blood lactate response was uniform for all diets. Similarly to the response of serum glucose, lactate concentration peaked immediately following RSET. As expected, lactate concentration returned to resting concentration by 24 h after RSET. Overall lactate concentration was different between mares and geldings, with mares having greater lactate concentration across all diets.

The accumulation of lactate following the RSET suggests that horses in the present study were utilizing anaerobic metabolism to meet the energy demand of exercise. Lactate concentration after RSET in the current study was similar to lactate concentration of 4.6 mmol/L reported by Hyyppä and others (1997) following submaximal exercise in the form of a 3,000 m of trotting. Phillips and others (2017) reported pre-exercise lactate concentrations similar to those in the present study and a concentration of 6.69 mmol/L following exercise designed to result in fatigue. When comparing the mean post-RSET lactate concentration of 3.59 mmol/L in the present study to results of previous research and to the accepted lactate threshold of 4 mmol/L (Marlin and Nankervis, 2002), it could be concluded that RSET in the current study was not sufficient to result in sustained, predominate anaerobic energy metabolism. It is important to note that exercise program prior to the start of the trial was not controlled for horses in the present study. It is possible that some horses may have had increased aerobic capacity due to differences in exercise prior to the study, resulting in decreased lactate accumulation of some horses relative to others.

Studies concerning gender differences in humans with respect to peak lactate have found similar results between males and females (Baumgart et al., 2014; Zhang et al. 1992). No differences in lactate response to exercise has been noted in previous research, though it is well documented that mares typically have a greater percentage of anaerobic type IIB fibers (Ronëus et al., 1991) than do stallions. A larger percentage of anaerobic functioning IIB fibers would theoretically lead to greater lactate accumulation in male horses compared to mares, and so the increased lactate in mares compared to geldings was an unexpected result.

## 4.6 Body Temperature

As expected, body temperature was increased immediately following RSET compared to immediately before RSET. All mean resting body temperatures were within the normal range for horses at rest (Marlin and Nankervis, 2006).

## 4.7 Respiratory Frequency

Respiratory frequency increased during the first 15 min of the RSET and peaked during the 8 min canter. During gallops, $R_f$ increased steadily and a decrease was observed again during the intermittent 2 min walks present between gallop bouts. A peak was seen during third and fourth gallop bouts.

It is possible that these increases during gallops was due to a decrease in stride length. As the horses became fatigued they may have decreased stride length and increased number of strides in order to maintain pace, which would correspond to the increased $R_f$ observed during canter and gallop bouts during RSET. Previous research has indicated horses are unable to alter $R_f$ independently of stride length (Butler et al., 1993).

Overall $R_f$ values across varying time points during the RSET were less for the present study than in previous reports (Art et al., 2006). This discrepancy was present during all gaits in the RSET. Horses in the present study were of a similar BW to horses used by Art et al. (2006), and so it is unlikely that size was a factor in these contradictory results. The discrepancy could be due to differences in exercise regimen and fitness among horses.

## 4.8 Tidal Volume

Tidal volume was greatest during the third gallop of RSET, and VT during the second gallop was intermediate between the third gallop and all other gaits. Horses may have been able to accommodate increased metabolic demands due to increased exercise intensity by altering VT during gallops. Mean VT across all diets were considerably less than values reported in previous studies during a similar exercise test (Art et al., 2006). Decreased VT values recorded in this study may have been the result of error within the Cosmed K4b2 respiratory mask, potentially due to the mask not ideally fitting the size and shape of horses' faces in the present study.

## 4.9 Oxygen consumption and carbon dioxide output

Overall $VO_2$ and $VCO_2$ remained relatively constant during the first 5 min of the RSET and increased as horses began trotting. Each increase of speed corresponded to an increased $VO_2$ and $VCO_2$. Because increased $VO_2$ and $VCO_2$ is indicative of increased metabolic activity in response to increased energy demand, it is expected that peaks of $VO_2$ and $VCO_2$ would occur during increased exercise intensity, and these results are supported by previously conducted research (Hanák et al., 2001; Marlin and Nankervis, 2002; Evans and Rose, 1987).

Oxygen intake and $VCO_2$ values obtained during the present study were less during all gaits compared to results obtained by Art and colleagues (2006) which also utilized the Cosmed K4b2 respiratory mask. Other studies have reported greater $VO_2$ and $VCO_2$ than those in the present study (Landgren et al., 1988; Hanák et al., 2001). Art and others (2006) reported that the K4b2 system underestimated $VCO_2$ at high speeds compared to a stationary respiratory mask commonly utilized in treadmill exercise tests.

It is possible that $VO_2$ and $VCO_2$ parameters were underestimated in the present study because of a potentially ill-fit due to varying size and shape of the horses' faces, particularly when considered with reduced VT values observed in the present study.

### 4.10 Heart Rate

Gait during RSET affected heart rate during exercise. As expected, increased HR relative to the beginning of the RSET was observed during all later gaits. A mean HR of 132, 130, and 135 beats/min over the entire RSET was observed for horses fed HQ, LQ, and LQ- respectively. These results indicate that the RSET utilized in the current study fall into the "heavy" to "very heavy" activity categories outlined in the NRC (2007). It is possible, however, that because horses in the present study were not conditioned for the RSET, they had elevated HR at slower speeds. Ridgeway (1994) suggested that unfit horses can display HR which are approximately 70 % greater than HR of horses performing identical exercise which have been conditioned to do so.

### 4.11 Conclusion

Performance horses are often subjected to intense exercise for relatively short intervals of time, and therefore glycogen metabolism is of particular interest to the equine industry. Relative to other species such as humans and rats, glycogen in horses is replenished at a much slower rate following exercise. Supplementation of different amounts of starch to horses has yielded inconclusive results in terms of glycogen utilization and replenishment. Given its large portion of the equine diet and regional variability, the present study sought to determine the role that quality of hay plays in glycogen metabolism during exercise.

The present study demonstrated that hay quality may not affect voluntary intake, as has been known to occur in ruminants. There is, however, an acclimation period during which horses become accustomed to different diets, and this should be considered during diet transitioning.

Results of this study suggest varying metabolic responses to exercise corresponding to sex. Geldings in the present study had uniform overall glycogen concentration regardless of diet, but varying glycogen concentrations of mares suggest that different sexes may store and metabolize glycogen differently in response to diet. Horses consuming HQ hay may have been more reliant on muscle glycogen as a source of energy during RSET. Consumption of LQ hay seemed to have a glycogen sparing effect in horses. Additionally, the contrast of glucose concentration of mares consuming HQ hay relative to horses consuming other diets suggests that these horses may be less reliant on other sources of energy during exercise.

Geldings in the present study seemed to be more reliant on anaerobic energy production, which is evidenced by their increased overall lactate concentration compared to mares. It is unclear whether this may have been a result of altered metabolism or whether muscle fiber type may have contributed to this difference, or both. Furthermore, while the overall lack of glycogen metabolism during the RSET seemed to indicate submaximal exercise, mean HR suggested that the horses in the present study were unfit and perhaps were reliant on other energy sources as fuel during exercise. Few conclusions can be drawn from the respiratory parameters in the present study due to their departure from normally observed physiological values, though the overall trends expressed during the RSET were expected.

The results of the current study suggest that consumption of LQ hay may result in a glycogen sparing effect in horses, and that metabolism during exercise may occur differently between mares and geldings. Further investigation is needed to elucidate the different energy substrates which may used in lieu of glycogen during exercise as a result of differences in hay quality.

Table 4.1 Nutrient analysis (DM basis) of concentrate, high quality hay, and low quality hay used in diets during the trial.

| | NDF, % | ADF, % | DM, % | ASH, % | ETHER-EXTRACT, % | CP, % |
|---|---|---|---|---|---|---|
| **NUTRENA SAFECHOICE ORIGINAL** | 38.24 | 15.82 | 86.51 | 10.11 | 6.91 | 14.20 |
| **HIGH QUALITY HAY** | 61.35 | 32.87 | 85.71 | 7.28 | 2.66 | 13.34 |
| **LOW QUALITY HAY** | 70.73 | 43.72 | 86.48 | 6.89 | 2.05 | 5.95 |

Table 4.2 Average body weight of horses consuming HQ, LQ, and LQ-[a] throughout the study.

| DIET | **Average BW (kg)** | **SD** |
|---|---|---|
| **HQ** | 496.0 | 15.39 |
| **LQ** | 498.9 | 67.10 |
| **LQ-** | 486.0 | 20.51 |

[a] HQ = high quality hay with concentrate balanced to meet energy requirement; LQ = low quality hay with concentrate balanced to meet energy requirement; LQ- = low quality hay with same amount of concentrate as HQ

Table 4.3 Hay intake by horses during the 7 d ad libitum access to hay period.

| **Day** | **Average hay intake, % BW** |
|---|---|
| **1** | 0.97[a] |
| **2** | 1.46[b] |
| **3** | 1.47[b] |
| **4** | 1.81[c] |
| **5** | 1.84[c] |
| **6** | 1.73[c] |
| **7** | 1.79[c] |
| **SEM** | 0.216 |
| **P-value** | < 0.0001 |

[a,b,c] means differ ($P < 0.05$)

Table 4.4 Daily nutrient intake during period in which horses were consuming concentrate (DM basis).

| Diet[a] | DMI, kg | NDF, % | ADF, % | Ash, % | Ether Extract, % | CP, % |
|---|---|---|---|---|---|---|
| **HQ** | 7.34 | 58.00[b] | 43.93[b] | 7.70 | 4.79 | 13.47[d] |
| **LQ** | 8.11 | 73.66[c] | 43.57[b] | 9.14 | 3.64 | 8.97[c] |
| **LQ-** | 7.29 | 76.41[d] | 45.94[c] | 8.67 | 3.21 | 8.24[b] |
| **SEM** | 0.624 | 0.145 | 0.305 | 1.380 | 0.356 | 0.072 |
| **P-value** | 0.3407 | < 0.0001 | < 0.0001 | 0.5686 | 0.5642 | < 0.0001 |

[a] HQ = high quality hay with concentrate balanced to meet energy requirement; LQ = low quality hay with concentrate balanced to meet energy requirement; LQ- = low quality hay with same amount of concentrate as HQ
[b,c,d] means differ (P < 0.05) within column

Table 4.5 Skeletal muscle glycogen concentrations of mares (n = 6) and geldings (n = 6) consuming each diet[a].

| | **Glycogen, μg/mg wet weight** | |
|---|---|---|
| **Diet** | Mares | Geldings |
| **HQ** | 3.73[b] | 5.29[c] |
| **LQ** | 4.30[bc] | 3.95[bc] |
| **LQ-** | 5.83[c] | 5.10[c] |
| **SEM** | 0.572 | 0.426 |
| **P-value** | 0.0243 | 0.0243 |

[a] HQ = high quality hay with concentrate balanced to meet energy requirement; LQ = low quality hay with concentrate balanced to meet energy requirement; LQ- = low quality hay with same amount of concentrate as HQ
c,d means differ (P < 0.05) within column and row

Table 4.6 Mean skeletal muscle glycogen concentration for each diet immediately before the ridden standardized exercise test (RSET), immediately after the RSET, and 24, 48, and 72 h after the RSET.

| | Glycogen, μg/mg wet weight | | | | |
|---|---|---|---|---|---|
| **Diet** | Pre | Post | 24 h | 48 h | 72 h |
| **HQ** | 5.01$^{c}$ | 3.37$^{b}$ | 5.33$^{c}$ | 4.63$^{bc}$ | 4.27$^{bc}$ |
| **LQ** | 3.49$^{bc}$ | 4.50$^{bc}$ | 3.90$^{bc}$ | 4.49$^{bc}$ | 3.82$^{bc}$ |
| **LQ** | 4.13$^{bc}$ | 5.66$^{c}$ | 5.33$^{c}$ | 6.44$^{c}$ | 5.60$^{c}$ |
| **SEM** | 0.829 | 0.769 | 0.828 | 0.828 | 0.929 |
| **P-value** | 0.0445 | 0.0445 | 0.0445 | 0.0445 | 0.0445 |

[a] HQ = high quality hay with concentrate balanced to meet energy requirement; LQ = low quality hay with concentrate balanced to meet energy requirement; LQ- = low quality hay with same amount of concentrate as HQ

[b,c] means differ ($P \leq 0.05$) within column and row

Table 4.7 Serum insulin concentration (mIU/L), blood lactate (mmol/L), and body temperature (°C) for horses fed HQ, LQ, and LQ- pooled across all sample times.

| | | | |
|---|---|---|---|
| **Diet[a]** | Insulin (mIU/L) | Lactate (mmol/L) | Body Temperature (°C) |
| **HQ** | 9.93 | 1.76 | 38.92 |
| **LQ** | 7.60 | 1.35 | 38.66 |
| **LQ-** | 11.54 | 0.89 | 38.51 |
| **SEM** | 4.398 | 0.422 | 0.619 |
| **P-value** | 0.7264 | 0.1525 | 0.3902 |

[a] HQ = high quality hay with concentrate balanced to meet energy requirement; LQ = low quality hay with concentrate balanced to meet energy requirement; LQ- = low quality hay with same amount of concentrate as HQ

Table 4.8 Serum glucose concentrations of mares (n = 6) and geldings (n = 6) consuming HQ, LQ, and LQ.

| | Concentration (mg/dL) | |
|---|---|---|
| **Diet**[a] | Mares | Geldings |
| **HQ** | 85.37[c] | 53.76[b] |
| **LQ** | 62.95[b] | 69.20[c] |
| **LQ-** | 58.49[b] | 68.46[c] |
| **SEM*** | 4.86 | 6.88 |
| **P-value** | 0.0001 | 0.0001 |

[a] HQ = high quality hay with concentrate balanced to meet energy requirement; LQ = low quality hay with concentrate balanced to meet energy requirement; LQ- = low quality hay with same amount of concentrate as HQ

[b, c] means differ ($P < 0.05$) within column

Table 4.9 Serum glucose concentration and blood lactate concentration of horses immediately before exercise (Pre), immediately after exercise (Post), and 24, 48, and 72 h after exercise.

| Sample Time | Glucose, mg/dL | Lactate, mmol/L |
|---|---|---|
| **Pre** | 56.02[a] | 0.73[a] |
| **Post** | 83.81[b] | 3.59[b] |
| **24 h** | 62.06[a] | 0.87[a] |
| **48 h** | 66.43[a] | 0.63[a] |
| **72 h** | 63.43[a] | 0.58[a] |
| **SEM** | 4.360 | 0.399 |
| **P-value** | 0.0009 | < 0.0001 |

[a, b] means differ ($P < 0.05$) within column

Table 4.10 Whole Blood Lactate (mmol/L) of mares (n = 6) and geldings (n = 6) consuming each diet pooled across all sampling times.

| Sex | Lactate (mg/dL) |
|---|---|
| **Mares** | 0.97[a] |
| **Geldings** | 1.70[b] |
| **SEM** | 0.335 |
| **P-value** | 0.0715 |

[a,b] means tend to differ ($0.1 < P \geq 0.05$)

Table 4.11 Body temperature of horses immediately before and after the ridden standardized exercise test.

| Sample Time | Body Temperature, $^{o}$C |
|---|---|
| **Pre** | 37.82[a] |
| **Post** | 39.46[b] |
| **SEM** | 0.1514 |
| **P-value** | < 0.0001 |

[a,b] Means differ ($P < 0.05$)

Table 4.12 Mean respiratory frequency (breaths/min) of horses during the different gaits of the ridden standardized exercise test.

| | HQ | LQ | LQ- |
|---|---|---|---|
| **Time During RSET** | **$R_f$, breaths/min** | **$R_f$, breaths/min** | **$R_f$, breaths/min** |
| **1st Walk** | 38.90$^{bc}$ | 29.95$^{b}$ | 38.44$^{bc}$ |
| **Trot** | 67.99$^{c}$ | 41.96$^{bc}$ | 70.21$^{cd}$ |
| **Canter** | 104.86$^{ef}$ | 78.74$^{cd}$ | 98.00$^{e}$ |
| **2nd Walk** | 94.87$^{de}$ | 89.72$^{de}$ | 89.62$^{de}$ |
| **1st Gallop** | 101.27$^{ef}$ | 119.03$^{ef}$ | 96.46$^{de}$ |
| **3rd Walk** | 95.07$^{de}$ | 88.74$^{d}$ | 100.51$^{ef}$ |
| **2nd Gallop** | 105.51$^{ef}$ | 118.65$^{ef}$ | 103.56$^{ef}$ |
| **4th Walk** | 97.68$^{dc}$ | 90.55$^{de}$ | 98.61$^{e}$ |
| **3rd Gallop** | 99.67$^{ef}$ | 122.26$^{f}$ | 101.01$^{ef}$ |
| **5th Walk** | 96.64$^{de}$ | 102.01$^{ef}$ | 97.84$^{de}$ |
| **4th Gallop** | 101.96$^{ef}$ | 59.71$^{c}$ | 99.15$^{ef}$ |
| **6th Walk** | 82.63$^{d}$ | 82.98$^{d}$ | 90.04$^{de}$ |
| **SEM** | 8.617 | 15.910 | 7.616 |
| **P-value** | 0.0043 | 0.0043 | 0.0043 |

[a] Gait: 1st walk = 1.5 m/s, trot = 4.5 m/s, canter = 5.5 m/s, 2nd walk = 1.5 m/s, 1st gallop = 6.5 m/s, 3rd walk = 1.5 m/s, 2nd gallop = 7.5 m/s, 4th walk = 1.5 m/s, 3rd gallop = 8.5 m/s, 5th walk = 1.5 m/s, 4th gallop = 9.5 m/s, 6th walk = 1.5 m/s

[b, c, d, e, f] means differ ($P < 0.05$) within column and row

Table 4.13 Mean tidal volume (VT), oxygen intake ($VCO_2$), carbon dioxide output ($VCO_2$), and heart rate (HR) of horses consuming all diets during each gait during the ridden standardized exercise test.

| Gait[a] | VT, liter/inspiration | $VO_2$, mL/min | $VCO_2$, mL/min | HR, beats/min |
|---|---|---|---|---|
| **1st Walk** | 3.20[b] | 62.9[b] | 62.4[b] | 66.3[b] |
| **Trot** | 0.67[b] | 391.5[cd] | 354.5[c] | 111.7[c] |
| **Canter** | 12.31[b] | 680.3[e] | 682.3[e] | 155.8[de] |
| **2nd Walk** | 23.16[b] | 766.3[e] | 694.5[e] | 164.9[de] |
| **1st Gallop** | 0.48[b] | 765.5[e] | 717.0[e] | 165.8[de] |
| **3rd Walk** | 0.43[b] | 586.9[de] | 605.3[e] | 131.2[cd] |
| **2nd Gallop** | 24.62[bc] | 372.8[cd] | 488.0[cd] | 174.0[e] |
| **4th Walk** | 0.43[b] | 386.3[cd] | 442.3[cd] | 122.2[cd] |
| **3rd Gallop** | 33.30[c] | 354.1[cd] | 464.5[cd] | 171.0[e] |
| **5th Walk** | 0.41[b] | 356.2[cd] | 409.4[cd] | 133.2[cd] |
| **4th Gallop** | 3.16[b] | 346.2[c] | 369.2[c] | 158.8[de] |
| **6th Walk** | 5.76[b] | 477.4[d] | 495.6[d] | 140.2[d] |
| **SEM** | 26.278 | 128.97 | 108.14 | 16.98 |
| **P-value** | 0.0445 | < 0.0001 | < 0.0001 | < 0.0001 |

[a] Gait: 1st walk = 1.5 m/s, trot = 4.5 m/s, canter = 5.5 m/s, 2nd walk = 1.5 m/s, 1st gallop = 6.5 m/s, 3rd walk = 1.5 m/s, 2nd gallop = 7.5 m/s, 4th walk = 1.5 m/s, 3rd gallop = 8.5 m/s, 5th walk = 1.5 m/s, 4th gallop = 9.5 m/s, 6th walk = 1.5 m/s

[b,c,d,e] means differ ($P < 0.05$) within column

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# APPENDIX A

## GRAPHICAL DEPICTION OF MEAN RESPIRATORY FREQUENCY OF HORSES CONSUMING HQ, LQ, AND LQ-, DURING A 44 MINUTE RIDDEN STANDARDIZED EXERCISE TEST

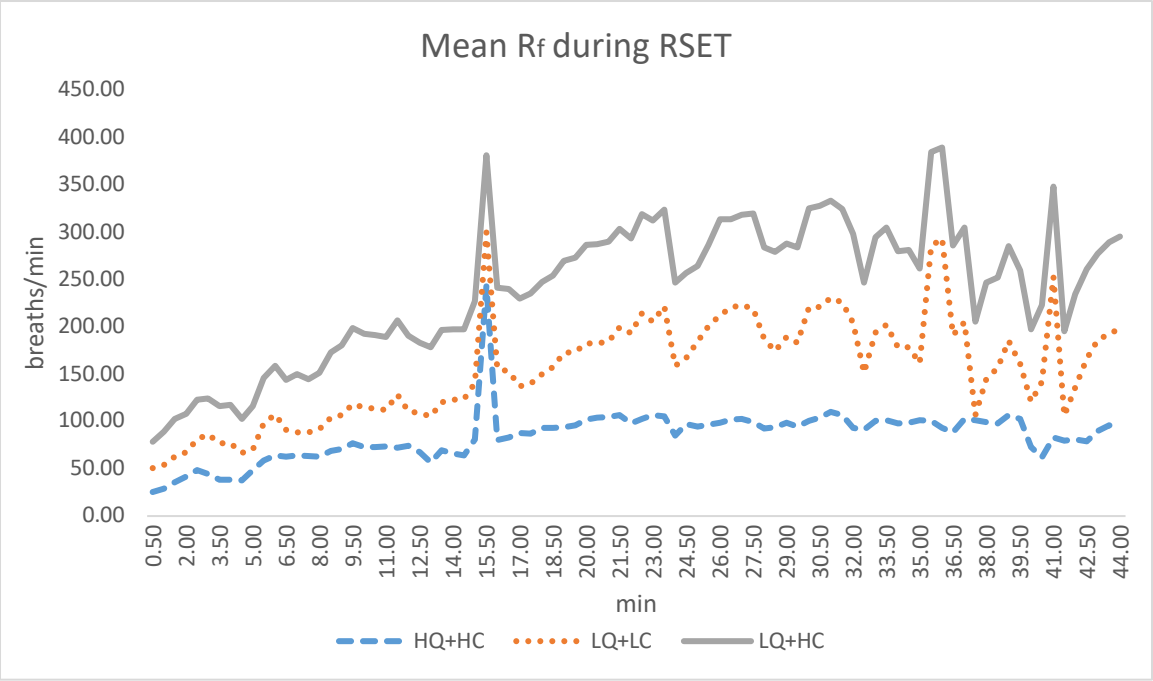


Figure A.1 Mean $R_f$ (breaths/min) of horses consuming HQ, LQ, and LQ- during a 44 min ridden standardized exercise test.

# APPENDIX B

# MEAN RESPIRATORY FREQUENCY FOR HORSES DURING A 44 MINUTE RIDDEN STANDARDIZED EXERCISE TEST

Table B.1 Mean respiratory frequency (breaths/min) for horses during a 44 min ridden standardized exercise test.

| | HQ | LQ | LQ- | | |
|---|---|---|---|---|---|
| **Time During RSET** | **$R_f$, breaths/min** | **$R_f$, breaths/min** | **$R_f$, breaths/min** | **SEM** | **P-value** |
| **0.50** | 25.44[a] | 25.25[a] | 27.86[a] | 18.547 | 0.8443 |
| **1.00** | 28.92[a] | 25.19[a] | 35.00[a] | 18.547 | 0.5592 |
| **1.50** | 36.03[a] | 26.75[a] | 40.08[a] | 18.547 | 0.4391 |
| **2.00** | 41.79[a] | 25.70[a] | 40.71[a] | 18.547 | 0.3875 |
| **2.50** | 48.84[a] | 32.19[a] | 42.10[a] | 18.547 | 0.5556 |
| **3.00** | 44.57[a] | 41.82[a] | 37.75[a] | 18.547 | 0.8702 |
| **3.50** | 38.42[a] | 38.25[a] | 39.52[a] | 23.268 | 0.9997 |
| **4.00** | 38.73[a] | 38.24[a] | 40.80[a] | 23.886 | 0.9076 |
| **4.50** | 37.70[a] | 28.89[a] | 36.13[a] | 24.539 | 0.7847 |
| **5.00** | 48.58[a] | 21.92[a] | 45.50[a] | 24.028 | 0.2018 |
| **5.50** | 58.58[a] | 39.21[a] | 48.18[a] | 19.507 | 0.6050 |
| **6.00** | 63.93[a] | 43.37[a] | 51.76[a] | 19.509 | 0.6250 |
| **6.50** | 62.90[a] | 27.97[a] | 53.26[a] | 24.050 | 0.3770 |
| **7.00** | 64.39[a] | 24.22[a] | 61.12[a] | 24.675 | 0.1367 |
| **7.50** | 63.46[a] | 25.11[a] | 56.24[a] | 24.789 | 0.1913 |
| **8.00** | 62.62[a] | 29.30[a] | 59.44[a] | 24.816 | 0.1985 |
| **8.50** | 69.11[a] | 35.09[a] | 69.08[a] | 24.823 | 0.1481 |
| **9.00** | 70.55[a] | 36.22[a] | 73.78[a] | 24.823 | 0.1121 |
| **9.50** | 77.15[a] | 41.27[a] | 80.08[a] | 24.516 | 0.1025 |
| **10.00** | 72.97[a] | 42.09[a] | 77.51[a] | 24.789 | 0.1398 |
| **10.50** | 72.72[a] | 40.70[a] | 77.62[a] | 24.675 | 0.1382 |
| **11.00** | 73.40[a] | 38.80[a] | 77.50[a] | 24.050 | 0.1610 |
| **11.50** | 71.99[a] | 56.44[a] | 78.19[a] | 19.510 | 0.2049 |

Table B.1 (continued)

| | | | | | |
|---|---|---|---|---|---|
| **12.00** | 74.21[a] | 36.55[a] | 79.56[a] | 24.050 | 0.1100 |
| **12.50** | 67.26[a] | 41.75[a] | 74.45[a] | 24.675 | 0.1898 |
| **13.00** | 56.95[a] | 48.36[a] | 73.37[a] | 24.788 | 0.2901 |
| **13.50** | 69.43[a] | 51.06[a] | 76.28[a] | 24.812 | 0.2778 |
| **14.00** | 65.85[a] | 56.80[a] | 74.94[a] | 24.810 | 0.4221 |
| **14.50** | 63.80[a] | 59.97[a] | 73.31[a] | 24.773 | 0.5503 |
| **15.00** | 81.04[a] | 59.71[a] | 86.56[a] | 24.573 | 0.2683 |
| **15.50** | 240.05[b] | 56.86[a] | 81.41[a] | 25.546 | < 0.0001 |
| **16.00** | 80.30[a] | 75.95[a] | 84.75[a] | 19.381 | 0.6390 |
| **16.50** | 83.10[a] | 69.69[a] | 86.88[a] | 19.491 | 0.3552 |
| **17.00** | 88.00[a] | 49.45[a] | 92.12[a] | 24.047 | 0.0878 |
| **17.50** | 87.19[a] | 52.80[a] | 95.04[a] | 24.671 | 0.0819 |
| **18.00** | 93.53[a] | 56.29[a] | 97.65[a] | 24.777 | 0.0837 |
| **18.50** | 93.31[a] | 63.98[a] | 96.86[a] | 24.775 | 0.1617 |
| **19.00** | 93.99[a] | 78.04[a] | 97.59[a] | 24.657 | 0.3870 |
| **19.50** | 95.65[a] | 77.99[a] | 99.78[a] | 23.945 | 0.3341 |
| **20.00** | 101.90[a] | 80.83[a] | 104.03[a] | 18.547 | 0.1921 |
| **20.50** | 104.12[a] | 80.83[a] | 104.03[a] | 23.943 | 0.2351 |
| **21.00** | 104.54[a] | 78.23[a] | 105.26[a] | 24.650 | 0.2731 |
| **21.50** | 106.66[a] | 80.19[a] | 105.21[a] | 24.747 | 0.5737 |
| **22.00** | 98.12[a] | 94.90[a] | 100.19[a] | 24.650 | 0.7259 |
| **22.50** | 102.57[a] | 111.54[a] | 104.99[a] | 23.943 | 0.9678 |
| **23.00** | 106.69[a] | 98.79[a] | 106.56[a] | 18.547 | 0.6355 |
| **23.50** | 105.54[a] | 115.96[a] | 102.40[a] | 23.946 | 0.7432 |
| **24.00** | 85.12[a] | 73.85[a] | 87.97[a] | 24.658 | 0.4691 |
| **24.50** | 97.44[a] | 69.36[a] | 90.12[a] | 24.772 | 0.3264 |
| **25.00** | 94.40[a] | 90.27[a] | 79.56[a] | 24.715 | 0.8198 |
| **25.50** | 96.71[a] | 104.06[a] | 86.13[a] | 24.074 | 0.5103 |
| **26.00** | 98.77[a] | 113.40[a] | 101.24[a] | 24.076 | 0.6748 |
| **26.50** | 101.81[a] | 118.44[a] | 93.31[a] | 24.722 | 0.3742 |
| **27.00** | 102.99[a] | 120.33[a] | 95.06[a] | 24.801 | 0.3615 |
| **27.50** | 99.55[a] | 119.71[a] | 100.62[a] | 24.787 | 0.5067 |

Table B.1 (continued)

| | | | | | |
|---|---|---|---|---|---|
| **28.00** | **92.41[a]** | **94.90[a]** | **96.62[a]** | **24.675** | **0.8439** |
| **28.50** | 93.95[a] | 80.27[a] | 105.25[a] | 24.050 | 0.2167 |
| **29.00** | 98.83[a] | 90.69[a] | 98.65[a] | 19.507 | 0.6359 |
| **29.50** | 94.46[a] | 88.42[a] | 100.96[a] | 19.491 | 0.4734 |
| **30.00** | 100.82[a] | 119.37[a] | 104.99[a] | 23.946 | 0.9125 |
| **30.50** | 104.02[a] | 117.13[a] | 106.72[a] | 23.921 | 0.8278 |
| **31.00** | 110.01[a] | 120.54[a] | 102.62[a] | 24.688 | 0.5753 |
| **31.50** | 106.55[a] | 119.40[a] | 98.46[a] | 24.800 | 0.4661 |
| **32.00** | 93.37[a] | 110.56[a] | 94.50[a] | 24.820 | 0.5825 |
| **32.50** | 91.30[a] | 59.86[a] | 95.52[a] | 24.822 | 0.1277 |
| **33.00** | 100.96[a] | 93.12[a] | 101.02[a] | 24.815 | 0.6834 |
| **33.50** | 101.32[a] | 100.55[a] | 102.89[a] | 24.787 | 0.8518 |
| **34.00** | 97.73[a] | . | 101.69[a] | 19.206 | 0.7809 |
| **34.50** | 98.35[a] | . | 102.57[a] | 19.322 | 0.7319 |
| **35.00** | 101.07[ab] | 60.23[a] | 100.30[b] | 18.547 | 0.0274 |
| **35.50** | 100.89[a] | 184.27[b] | 99.32[a] | 19.381 | <0.0001 |
| **36.00** | 93.36[a] | 200.00[b] | 96.13[a] | 24.035 | 0.0036 |
| **36.50** | 88.86[a] | . | 95.12[a] | 19.473 | 0.8207 |
| **37.00** | 102.40[a] | . | 100.33[a] | 19.491 | 0.9662 |
| **37.50** | 101.50[b] | 4.95[a] | 99.26[b] | 24.812 | 0.0001 |
| **38.00** | 99.52[b] | 45.38[a] | 102.02[b] | 24.812 | 0.0185 |
| **38.50** | 97.96[a] | 58.99[a] | 95.37[a] | 24.785 | 0.1349 |
| **39.00** | 106.44[a] | 78.51[a] | 100.00[a] | 24.660 | 0.4330 |
| **39.50** | 102.95[a] | 57.83[a] | 98.67[a] | 23.946 | 0.1964 |
| **40.00** | 72.94[a] | 48.23[a] | 75.91[a] | 18.547 | 0.1226 |
| **40.50** | 62.29[a] | 79.71[a] | 80.93[a] | 23.725 | 0.4810 |
| **41.00** | 83.30[a] | 169.48[b] | 95.51[a] | 24.572 | 0.0025 |
| **41.50** | 79.44[b] | 26.91[a] | 88.75[b] | 24.771 | 0.0116 |
| **42.00** | 80.96[a] | 54.77[a] | 98.52[a] | 26.068 | 0.0818 |
| **42.50** | 79.31[a] | 86.65[a] | 94.81[a] | 26.051 | 0.6297 |
| **43.00** | 90.15[a] | 95.62[a] | 91.10[a] | 24.674 | 0.9717 |
| **43.50** | 96.14[a] | 96.18[a] | 97.06[a] | 24.050 | 0.7619 |

Table B.1 (continued)

| | | | | | |
|---|---|---|---|---|---|
| **44.00** | **93.13[a]** | **104.68[a]** | **97.43[a]** | **19.510** | **0.7559** |

[a, b] means differ ($P < 0.05$) within row

# APPENDIX C

## GRAPHICAL DEPICTION OF MEAN TIDAL VOLUME OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE RIDDEN STANDARDIZED EXERCISE TEST

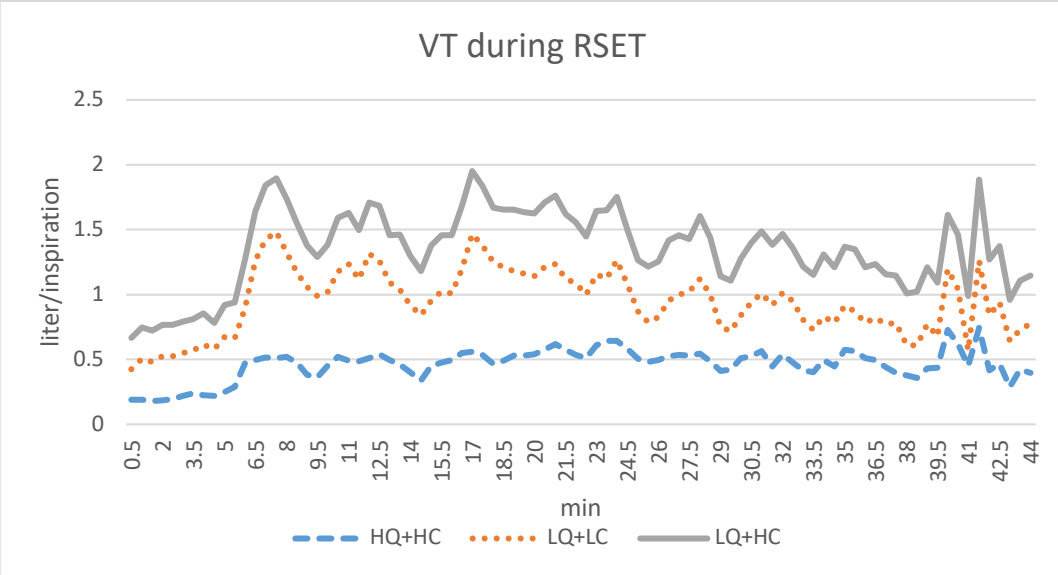


Figure C.1 Mean VT (liter/inspiration) of horses consuming HQ, LQ, and LQ- during a 44 min ridden standardized exercise test.

# APPENDIX D

# MEAN TIDAL VOLUME OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE RIDDEN STANDARDIZED EXERCISE TEST

Table D.1 Mean VT (liter/inspiration) for horses consuming HQ, LQ, and LQ- during the 44 min ridden standardized exercise test.

| | HQ | LQ | LQ- |
|---|---|---|---|
| **Time During RSET** | **Mean VT** | **Mean VT** | **Mean VT** |
| **0.50** | 0.19 | 0.23 | 4.60 |
| **1.00** | 0.19 | 0.31 | 9.66 |
| **1.50** | 0.17 | 0.30 | 9.09 |
| **2.00** | 0.18 | 0.34 | 8.01 |
| **2.50** | 0.19 | 0.32 | 5.68 |
| **3.00** | 0.21 | 0.33 | 10.61 |
| **3.50** | 0.24 | 0.32 | 7.34 |
| **4.00** | 0.22 | 0.38 | 8.98 |
| **4.50** | 0.22 | 0.35 | 0.20 |
| **5.00** | 0.24 | 0.42 | 0.24 |
| **5.50** | 0.28 | 0.37 | 0.27 |
| **6.00** | 0.46 | 0.42 | 0.37 |
| **6.50** | 0.49 | 0.76 | 0.37 |
| **7.00** | 0.51 | 0.90 | 0.41 |
| **7.50** | 0.51 | 0.97 | 0.41 |
| **8.00** | 0.52 | 0.80 | 0.41 |
| **8.50** | 0.47 | 0.69 | 0.38 |
| **9.00** | 0.38 | 0.67 | 0.31 |
| **9.50** | 0.36 | 0.62 | 0.30 |
| **10.00** | 0.45 | 0.56 | 0.36 |
| **10.50** | 0.52 | 0.65 | 0.41 |
| **11.00** | 0.49 | 0.74 | 7.15 |
| **11.50** | 0.48 | 0.63 | 0.37 |
| **12.00** | 0.51 | 0.79 | 0.39 |

Table D.11 (continued)

| | | | |
|---|---|---|---|
| **12.50** | **0.54** | **0.71** | **0.42** |
| **13.00** | 0.49 | 0.59 | 0.36 |
| **13.50** | 0.46 | 0.57 | 0.42 |
| **14.00** | 0.40 | 0.51 | 0.38 |
| **14.50** | 0.33 | 0.50 | 0.33 |
| **15.00** | 0.45 | 0.50 | 0.42 |
| **15.50** | 235.48 | 0.54 | 10.31 |
| **16.00** | 0.49 | 0.51 | 0.44 |
| **16.50** | 0.54 | 0.64 | 0.48 |
| **17.00** | 0.55 | 0.91 | 0.48 |
| **17.50** | 0.53 | 0.84 | 0.46 |
| **18.00** | 0.46 | 0.78 | 0.41 |
| **18.50** | 0.48 | 0.72 | 0.44 |
| **19.00** | 0.52 | 0.65 | 0.47 |
| **19.50** | 0.53 | 0.63 | 0.47 |
| **20.00** | 0.54 | 0.59 | 95.68 |
| **20.50** | 0.57 | 0.63 | 0.49 |
| **21.00** | 0.61 | 0.61 | 0.52 |
| **21.50** | 0.57 | 0.54 | 0.49 |
| **22.00** | 0.53 | 0.55 | 0.47 |
| **22.50** | 0.50 | 0.49 | 0.44 |
| **23.00** | 0.60 | 0.55 | 104.97 |
| **23.50** | 0.64 | 0.47 | 0.52 |
| **24.00** | 0.64 | 0.62 | 0.48 |
| **24.50** | 0.57 | 0.49 | 0.41 |
| **25.00** | 0.50 | 0.36 | 0.39 |
| **25.50** | 0.48 | 0.30 | 82.40 |
| **26.00** | 0.49 | 0.33 | 105.67 |
| **26.50** | 0.52 | 0.42 | 0.46 |
| **27.00** | 0.53 | 0.46 | 0.45 |
| **27.50** | 0.52 | 0.49 | 0.40 |
| **28.00** | 0.54 | 0.57 | 0.48 |

Table D.11 (continued)

| | | | |
|---|---|---|---|
| **28.50** | **0.48** | **0.51** | **0.43** |
| **29.00** | 0.41 | 0.33 | 0.39 |
| **29.50** | 0.42 | 0.29 | 0.39 |
| **30.00** | 0.50 | 0.33 | 0.43 |
| **30.50** | 0.52 | 0.41 | 151.41 |
| **31.00** | 0.56 | 0.44 | 0.48 |
| **31.50** | 0.44 | 0.47 | 0.46 |
| **32.00** | 0.53 | 0.47 | 0.45 |
| **32.50** | 0.47 | 0.47 | 0.40 |
| **33.00** | 0.41 | 0.38 | 0.40 |
| **33.50** | 0.39 | 0.33 | 0.42 |
| **34.00** | 0.49 | . | 0.47 |
| **34.50** | 0.44 | . | 0.42 |
| **35.00** | 0.57 | 0.34 | 138.43 |
| **35.50** | 0.56 | 134.83 | 0.47 |
| **36.00** | 0.51 | 0.27 | 0.42 |
| **36.50** | 0.49 | . | 0.41 |
| **37.00** | 0.44 | . | 0.38 |
| **37.50** | 0.39 | 0.38 | 0.36 |
| **38.00** | 0.37 | 0.23 | 0.39 |
| **38.50** | 0.35 | 0.24 | 23.52 |
| **39.00** | 0.42 | 0.34 | 0.43 |
| **39.50** | 0.43 | 0.24 | 0.41 |
| **40.00** | 0.72 | 0.46 | 53.27 |
| **40.50** | 0.61 | 0.42 | 22.00 |
| **41.00** | 0.45 | 0.11 | 0.41 |
| **41.50** | 0.74 | 0.51 | 0.62 |
| **42.00** | 0.41 | 0.42 | 0.42 |
| **42.50** | 0.46 | 0.47 | 0.43 |
| **43.00** | 0.29 | 0.35 | 0.31 |
| **43.50** | 0.42 | 0.30 | 0.38 |
| **44.00** | 0.39 | 0.38 | 0.36 |

# APPENDIX E

## GRAPHICAL DEPICITON OF MEAN $VO_2$ OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE STANDARDIZED EXERCISE TEST

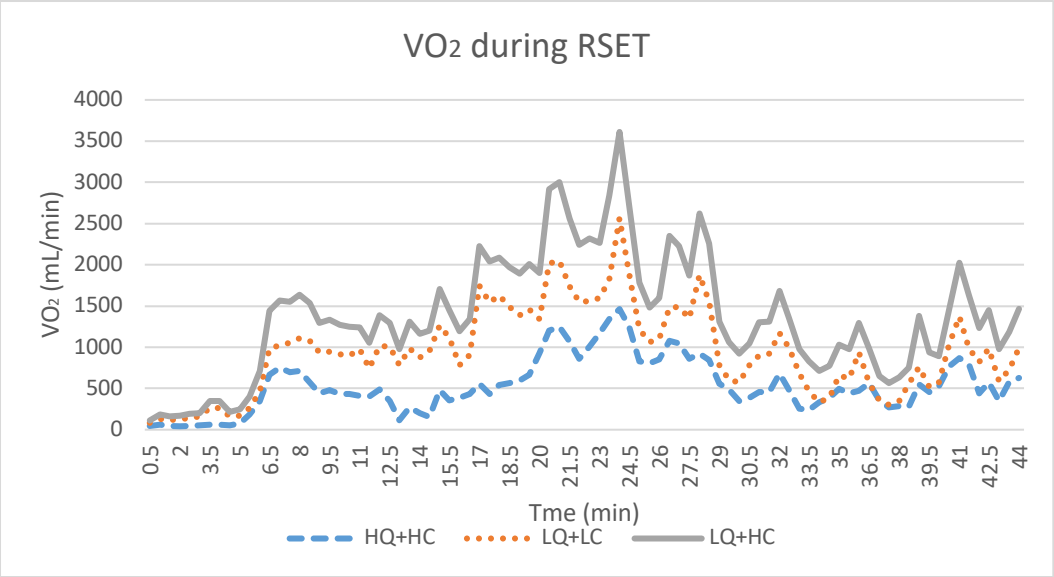


Figure E.1 Mean $VO_2$ (mL/min) of horses consuming HQ, LQ, and LQ- during a 44 min ridden standardized exercise test.

# APPENDIX F

# MEAN $VO_2$ OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE STANDARDIZED EXERCISE TEST

Table F.1 Mean $VO_2$ (mL/min) for horses consuming HQ, LQ, and LQ- during the 44 min ridden standardized exercise test.

| | HQ | LQ | LQ- |
|---|---|---|---|
| **Time During RSET** | **Mean $VO_2$** | **Mean $VO_2$** | **Mean $VO_2$** |
| **0.50** | 47.82 | 35.71 | 28.75 |
| **1.00** | 58.95 | 73.79 | 51.66 |
| **1.50** | 48.06 | 65.85 | 47.91 |
| **2.00** | 41.32 | 92.67 | 34.13 |
| **2.50** | 42.96 | 95.46 | 54.07 |
| **3.00** | 50.92 | 108.08 | 39.58 |
| **3.50** | 58.45 | 207.91 | 83.79 |
| **4.00** | 62.39 | 202.49 | 84.61 |
| **4.50** | 52.86 | 105.45 | 59.44 |
| **5.00** | 74.83 | 95.92 | 80.09 |
| **5.50** | 182.63 | 81.83 | 141.03 |
| **6.00** | 348.21 | 113.72 | 248.67 |
| **6.50** | 667.75 | 316.20 | 462.16 |
| **7.00** | 753.94 | 271.51 | 541.69 |
| **7.50** | 697.55 | 358.57 | 497.66 |
| **8.00** | 713.94 | 397.49 | 523.18 |
| **8.50** | 576.64 | 502.84 | 456.94 |
| **9.00** | 442.88 | 491.34 | 357.16 |
| **9.50** | 478.28 | 470.17 | 387.91 |
| **10.00** | 434.50 | 483.49 | 354.69 |
| **10.50** | 436.29 | 460.08 | 352.14 |
| **11.00** | 413.40 | 565.68 | 261.78 |
| **11.50** | 400.71 | 340.67 | 311.67 |

Table F.11 (continued)

| | | | |
|---|---|---|---|
| **12.00** | 490.76 | 523.09 | 374.54 |
| **12.50** | 349.91 | 656.87 | 288.36 |
| **13.00** | 113.07 | 670.15 | 190.97 |
| **13.50** | 270.09 | 714.86 | 327.78 |
| **14.00** | 200.43 | 677.48 | 283.47 |
| **14.50** | 152.52 | 803.53 | 242.94 |
| **15.00** | 478.80 | 788.11 | 439.77 |
| **15.50** | 353.59 | 765.01 | 328.75 |
| **16.00** | 385.55 | 396.71 | 414.66 |
| **16.50** | 435.17 | 469.14 | 440.11 |
| **17.00** | 556.67 | 1185.27 | 488.08 |
| **17.50** | 432.82 | 1110.69 | 498.68 |
| **18.00** | 542.67 | 1086.16 | 458.90 |
| **18.50** | 563.64 | 926.07 | 483.95 |
| **19.00** | 599.51 | 785.89 | 510.23 |
| **19.50** | 663.83 | 790.90 | 556.45 |
| **20.00** | 932.55 | 411.76 | 554.27 |
| **20.50** | 1198.62 | 815.48 | 904.95 |
| **21.00** | 1248.31 | 800.44 | 956.69 |
| **21.50** | 1068.86 | 671.77 | 817.47 |
| **22.00** | 863.75 | 690.63 | 688.00 |
| **22.50** | 1011.27 | 546.49 | 764.39 |
| **23.00** | 1170.38 | 430.95 | 666.96 |
| **23.50** | 1339.08 | 486.88 | 1015.67 |
| **24.00** | 1462.25 | 1103.91 | 1045.47 |
| **24.50** | 1251.11 | 643.13 | 829.99 |
| **25.00** | 828.95 | 388.77 | 569.16 |
| **25.50** | 805.84 | 254.65 | 417.69 |
| **26.00** | 852.94 | 248.22 | 497.91 |
| **26.50** | 1076.13 | 404.55 | 867.76 |
| **27.00** | 1045.49 | 441.23 | 739.61 |
| **27.50** | 858.54 | 493.00 | 521.36 |

Table F.11 (continued)

| | | | |
|---|---|---|---|
| **28.00** | 923.60 | 953.84 | 742.02 |
| **28.50** | 844.11 | 710.18 | 704.68 |
| **29.00** | 555.95 | 223.96 | 527.78 |
| **29.50** | 489.95 | 116.97 | 454.06 |
| **30.00** | 350.35 | 218.93 | 353.10 |
| **30.50** | 383.35 | 395.62 | 271.12 |
| **31.00** | 455.46 | 445.64 | 403.88 |
| **31.50** | 455.12 | 470.49 | 381.37 |
| **32.00** | 681.46 | 490.46 | 511.47 |
| **32.50** | 476.87 | 514.19 | 357.46 |
| **33.00** | 252.01 | 427.49 | 293.46 |
| **33.50** | 329.65 | 154.35 | 383.05 |
| **34.00** | 387.12 | . | 386.10 |
| **34.50** | 498.05 | . | 386.10 |
| **35.00** | 498.05 | 157.22 | 372.00 |
| **35.50** | 442.30 | 178.59 | 357.32 |
| **36.00** | 473.24 | 468.03 | 356.34 |
| **36.50** | 569.12 | . | 416.38 |
| **37.00** | 359.44 | . | 290.12 |
| **37.50** | 271.94 | 27.15 | 268.29 |
| **38.00** | 283.30 | 68.46 | 283.93 |
| **38.50** | 276.96 | 276.96 | 195.77 |
| **39.00** | 546.26 | 213.41 | 622.42 |
| **39.50** | 453.70 | 67.91 | 417.88 |
| **40.00** | 528.07 | 43.53 | 323.30 |
| **40.50** | 766.27 | 268.46 | 422.20 |
| **41.00** | 867.14 | 504.73 | 652.54 |
| **41.50** | 787.61 | 193.06 | 646.43 |
| **42.00** | 443.43 | 378.93 | 413.42 |
| **42.50** | 570.94 | 414.76 | 466.95 |
| **43.00** | 350.13 | 239.51 | 384.68 |
| **43.50** | 577.33 | 154.73 | 454.88 |

Table F.11 (continued)

| | | | |
|---|---|---|---|
| **44.00** | 630.13 | 362.09 | 474.91 |

# APPENDIX G

## GRAPHICAL DEPICITON OF MEAN $VCO_2$ OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE STANDARDIZED EXERCISE TEST

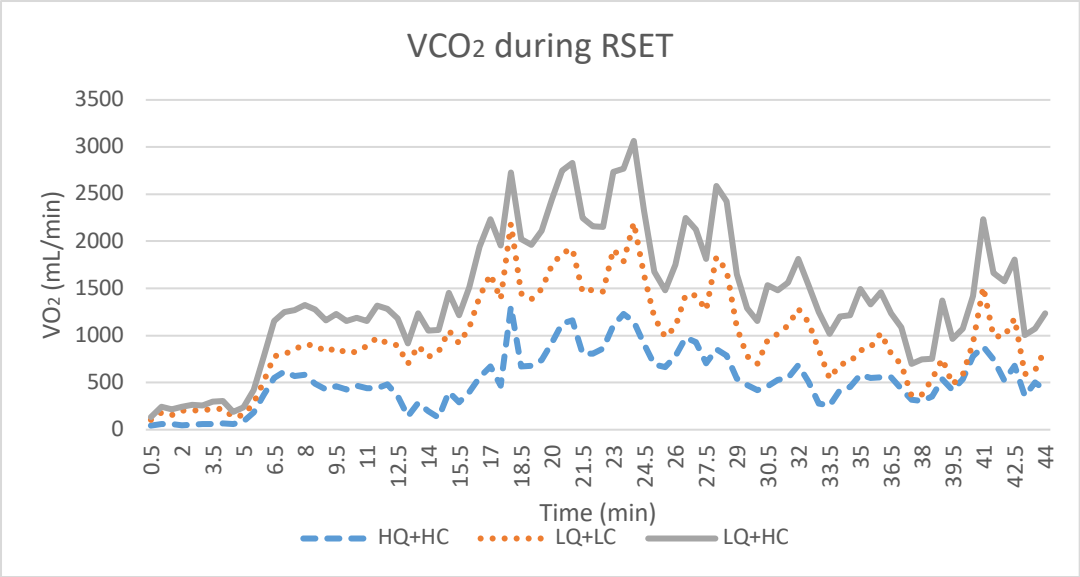


Figure G.1 Mean $VCO_2$ (mL/min) of horses consuming HQ, LQ, and LQ- during a 44 min ridden standardized exercise test.

# APPENDIX H

# MEAN $VCO_2$ OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE STANDARDIZED EXERCISE TEST

Table H.1 Mean $VCO_2$ (mL/min) of horses consuming HQ, LQ, and LQ- during 44 min ridden standardized exercise test.

| | HQ | LQ | LQ- |
|---|---|---|---|
| **Time During RSET** | **Mean $VCO_2$** | **Mean $VCO_2$** | **Mean $VCO_2$** |
| **0.50** | 43.53 | 61.28 | 29.28 |
| **1.00** | 61.55 | 120.21 | 58.82 |
| **1.50** | 57.43 | 99.80 | 55.85 |
| **2.00** | 49.51 | 150.75 | 42.56 |
| **2.50** | 55.00 | 148.08 | 57.38 |
| **3.00** | 58.17 | 148.54 | 52.20 |
| **3.50** | 61.63 | 154.40 | 82.87 |
| **4.00** | 65.84 | 156.19 | 83.44 |
| **4.50** | 58.66 | 76.84 | 55.04 |
| **5.00** | 87.01 | 69.11 | 82.79 |
| **5.50** | 184.60 | 99.55 | 137.61 |
| **6.00** | 374.04 | 143.50 | 259.73 |
| **6.50** | 548.14 | 229.00 | 374.51 |
| **7.00** | 614.28 | 188.33 | 444.88 |
| **7.50** | 572.30 | 285.23 | 409.07 |
| **8.00** | 579.77 | 320.02 | 423.06 |
| **8.50** | 489.37 | 404.69 | 380.95 |
| **9.00** | 424.22 | 405.61 | 332.81 |
| **9.50** | 458.60 | 408.74 | 360.91 |
| **10.00** | 426.89 | 388.71 | 338.87 |
| **10.50** | 464.60 | 361.15 | 364.87 |
| **11.00** | 440.71 | 449.32 | 260.52 |
| **11.50** | 440.30 | 537.36 | 337.07 |
| **12.00** | 480.54 | 435.32 | 365.07 |

Table H.11 (continued)

| | | | |
|---|---|---|---|
| **12.50** | **357.17** | **538.60** | **286.93** |
| **13.00** | 142.102 | 557.84 | 212.49 |
| **13.50** | 287.05 | 604.80 | 341.06 |
| **14.00** | 192.72 | 578.65 | 278.73 |
| **14.50** | 129.28 | 699.19 | 227.94 |
| **15.00** | 400.74 | 652.34 | 396.71 |
| **15.50** | 291.85 | 625.45 | 299.79 |
| **16.00** | 401.79 | 686.17 | 422.49 |
| **16.50** | 557.15 | 856.22 | 530.99 |
| **17.00** | 672.55 | 978.32 | 582.80 |
| **17.50** | 467.24 | 914.85 | 570.44 |
| **18.00** | 1305.55 | 880.17 | 546.42 |
| **18.50** | 672.66 | 774.68 | 577.61 |
| **19.00** | 678.49 | 702.62 | 580.00 |
| **19.50** | 740.42 | 750.30 | 619.96 |
| **20.00** | 923.68 | 818.78 | 703.66 |
| **20.50** | 1124.36 | 748.81 | 873.16 |
| **21.00** | 1158.86 | 761.48 | 913.98 |
| **21.50** | 808.98 | 646.85 | 791.88 |
| **22.00** | 805.77 | 681.15 | 673.03 |
| **22.50** | 863.75 | 600.61 | 687.38 |
| **23.00** | 1107.85 | 808.24 | 818.60 |
| **23.50** | 1230.80 | 554.33 | 982.31 |
| **24.00** | 1145.21 | 1053.11 | 866.52 |
| **24.50** | 911.74 | 731.49 | 677.57 |
| **25.00** | 693.26 | 501.39 | 478.75 |
| **25.50** | 667.13 | 317.07 | 493.92 |
| **26.00** | 776.81 | 312.25 | 664.99 |
| **26.50** | 978.15 | 455.89 | 812.41 |
| **27.00** | 930.00 | 494.36 | 702.21 |
| **27.50** | 702.50 | 563.44 | 547.43 |
| **28.00** | 855.40 | 975.31 | 757.19 |

Table H.11 (continued)

| | | | |
|---|---|---|---|
| **28.50** | **786.94** | **902.90** | **733.28** |
| **29.00** | 540.61 | 541.56 | 570.45 |
| **29.50** | 473.47 | 308.16 | 505.11 |
| **30.00** | 416.95 | 283.25 | 453.92 |
| **30.50** | 463.86 | 488.99 | 584.05 |
| **31.00** | 530.59 | 485.47 | 464.22 |
| **31.50** | 545.62 | 557.43 | 460.01 |
| **32.00** | 686.60 | 594.45 | 534.67 |
| **32.50** | 506.61 | 630.52 | 397.50 |
| **33.00** | 277.11 | 571.81 | 400.50 |
| **33.50** | 256.57 | 282.95 | 477.43 |
| **34.00** | 420.62 | . | 514.75 |
| **34.50** | 451.12 | . | 495.51 |
| **35.00** | 584.71 | 253.51 | 655.91 |
| **35.50** | 546.19 | 330.01 | 456.68 |
| **36.00** | 552.44 | 473.81 | 431.37 |
| **36.50** | 554.73 | . | 421.94 |
| **37.00** | 436.37 | . | 394.44 |
| **37.50** | 320.57 | 36.99 | 341.49 |
| **38.00** | 303.81 | 70.40 | 369.46 |
| **38.50** | 348.65 | 185.74 | 221.71 |
| **39.00** | 537.85 | 215.49 | 614.38 |
| **39.50** | 417.97 | 63.41 | 481.51 |
| **40.00** | 533.42 | 47.79 | 492.24 |
| **40.50** | 781.80 | 139.19 | 498.99 |
| **41.00** | 882.61 | 629.16 | 723.95 |
| **41.50** | 743.82 | 245.85 | 676.11 |
| **42.00** | 520.07 | 472.66 | 583.87 |
| **42.50** | 681.28 | 501.16 | 626.39 |
| **43.00** | 360.60 | 229.04 | 416.42 |
| **43.50** | 502.53 | 128.90 | 443.18 |
| **44.00** | 422.61 | 430.89 | 383.76 |

# APPENDIX I

## GRAPHICAL DEPICTION OF MEAN HEART RATE OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE STANDARDIZED EXERCISE TEST

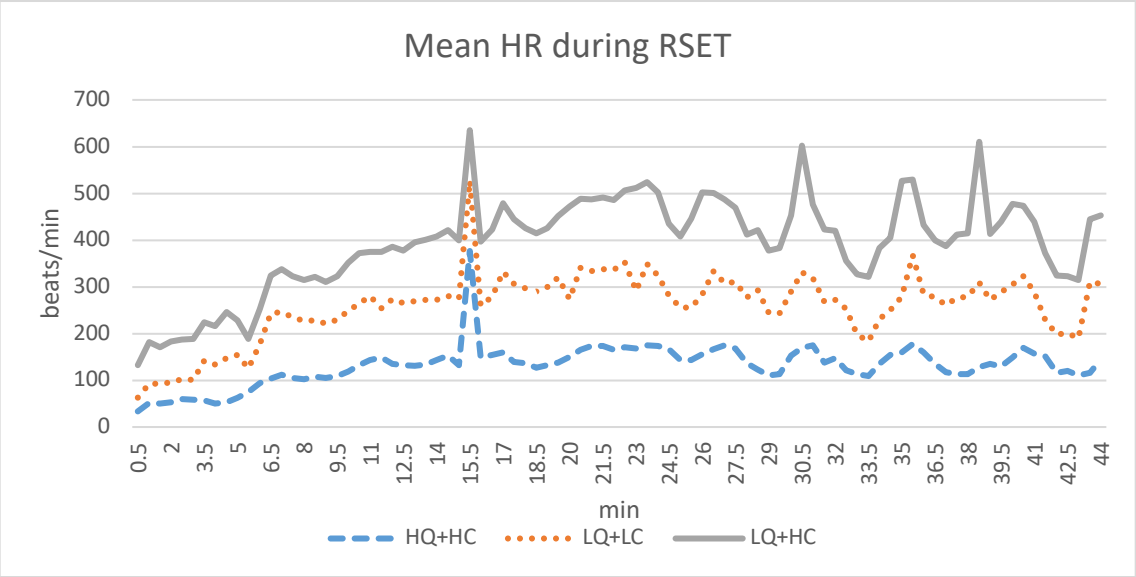


Figure I.1 Mean HR (beats/min) of horses consuming HQ, LQ, and LQ- diets during a 44 min ridden standardized exercise test

# APPENDIX J

# MEAN HEART RATE OF HORSES CONSUMING HQ, LQ, AND LQ- DURING A 44 MINUTE STANDARDIZED EXERCISE TEST

Table J.1 Mean HR (beats/min) of horses consuming HQ, LQ, and LQ- during the 44 min ridden standardized exercise test.

| | HQ | LQ | LQ- |
|---|---|---|---|
| **Time During RSET** | **Mean HR** | **Mean HR** | **Mean HR** |
| **0.50** | 33.49 | 29.38 | 69.52 |
| **1.00** | 51.12 | 41.72 | 88.26 |
| **1.50** | 49.64 | 43.07 | 77.42 |
| **2.00** | 52.79 | 42.03 | 87.76 |
| **2.50** | 60.29 | 42.40 | 84.73 |
| **3.00** | 58.50 | 43.00 | 87.15 |
| **3.50** | 56.99 | 85.27 | 82.07 |
| **4.00** | 49.53 | 84.61 | 82.05 |
| **4.50** | 52.71 | 95.07 | 98.22 |
| **5.00** | 62.28 | 92.00 | 73.31 |
| **5.50** | 75.06 | 50.41 | 62.43 |
| **6.00** | 93.93 | 83.83 | 75.13 |
| **6.50** | 103.24 | 139.50 | 81.17 |
| **7.00** | 111.53 | 135.16 | 91.46 |
| **7.50** | 104.83 | 130.50 | 87.48 |
| **8.00** | 102.03 | 125.40 | 87.19 |
| **8.50** | 107.56 | 121.05 | 92.15 |
| **9.00** | 104.50 | 117.07 | 89.40 |
| **9.50** | 108.96 | 120.88 | 93.06 |
| **10.00** | 119.26 | 130.05 | 102.35 |
| **10.50** | 131.83 | 131.85 | 108.95 |
| **11.00** | 143.80 | 136.15 | 94.78 |
| **11.50** | 148.92 | 105.51 | 120.61 |
| **12.00** | 134.89 | 138.83 | 111.49 |

Table J.11 (continued)

| | | | |
|---|---|---|---|
| **12.50** | **132.23** | **133.57** | **111.06** |
| **13.00** | 130.42 | 139.50 | 125.53 |
| **13.50** | 133.82 | 138.60 | 127.90 |
| **14.00** | 142.71 | 129.37 | 135.83 |
| **14.50** | 153.16 | 127.20 | 141.01 |
| **15.00** | 132.74 | 142.06 | 125.14 |
| **15.50** | 376.58 | 150.57 | 108.05 |
| **16.00** | 148.44 | 111.28 | 136.36 |
| **16.50** | 154.11 | 125.38 | 142.73 |
| **17.00** | 159.85 | 172.60 | 146.39 |
| **17.50** | 138.61 | 167.40 | 138.76 |
| **18.00** | 136.19 | 161.07 | 128.45 |
| **18.50** | 127.40 | 162.71 | 124.48 |
| **19.00** | 132.82 | 166.30 | 126.73 |
| **19.50** | 138.25 | 181.41 | 131.31 |
| **20.00** | 149.66 | 123.41 | 198.64 |
| **20.50** | 165.84 | 174.78 | 148.50 |
| **21.00** | 173.09 | 160.63 | 153.13 |
| **21.50** | 173.08 | 165.22 | 152.48 |
| **22.00** | 164.60 | 172.46 | 148.37 |
| **22.50** | 171.32 | 181.89 | 152.56 |
| **23.00** | 167.66 | 123.60 | 219.95 |
| **23.50** | 174.34 | 173.82 | 175.51 |
| **24.00** | 173.08 | 151.60 | 177.51 |
| **24.50** | 166.31 | 114.67 | 154.11 |
| **25.00** | 143.61 | 112.00 | 152.57 |
| **25.50** | 143.90 | 111.82 | 190.21 |
| **26.00** | 156.14 | 126.73 | 219.71 |
| **26.50** | 167.06 | 166.16 | 167.97 |
| **27.00** | 174.53 | 133.08 | 179.78 |
| **27.50** | 168.55 | 141.20 | 159.02 |
| **28.00** | 136.23 | 141.15 | 133.91 |

Table J.11 (continued)

| | | | |
|---|---|---|---|
| **28.50** | **123.32** | **169.87** | **127.50** |
| **29.00** | 110.14 | 133.95 | 133.92 |
| **29.50** | 112.91 | 130.76 | 139.90 |
| **30.00** | 152.82 | 137.54 | 162.08 |
| **30.50** | 169.81 | 161.67 | 270.10 |
| **31.00** | 174.69 | 143.96 | 157.91 |
| **31.50** | 138.40 | 130.10 | 154.64 |
| **32.00** | 147.84 | 123.60 | 148.79 |
| **32.50** | 121.12 | 131.64 | 103.36 |
| **33.00** | 113.63 | 84.34 | 129.04 |
| **33.50** | 109.60 | 73.08 | 138.02 |
| **34.00** | 134.64 | . | 152.82 |
| **34.50** | 154.37 | . | 154.98 |
| **35.00** | 159.36 | 118.93 | 248.33 |
| **35.50** | 177.95 | 190.41 | 161.68 |
| **36.00** | 160.32 | 124.00 | 147.80 |
| **36.50** | 134.46 | . | 121.84 |
| **37.00** | 117.99 | . | 125.20 |
| **37.50** | 113.04 | 162.66 | 135.75 |
| **38.00** | 113.06 | 166.00 | 135.44 |
| **38.50** | 128.07 | 181.00 | 301.65 |
| **39.00** | 134.84 | 137.75 | 140.15 |
| **39.50** | 129.92 | 159.00 | 150.71 |
| **40.00** | 149.44 | 155.16 | 173.54 |
| **40.50** | 169.37 | 152.80 | 151.25 |
| **41.00** | 157.50 | 131.00 | 150.97 |
| **41.50** | 150.87 | 79.20 | 141.67 |
| **42.00** | 115.57 | 82.00 | 127.01 |
| **42.50** | 120.3 | 80.78 | 121.85 |
| **43.00** | 110.37 | 79.75 | 124.01 |
| **43.50** | 116.40 | 193.64 | 134.80 |
| **44.00** | 144.55 | 161.35 | 146.29 |