THE EFFECT OF DIETARY STARCH CONCENTRATION ON GLYCOGEN

REPLENISHMENT IN PERFORMANCE HORSES

A Thesis

by

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ABSTRACT

Four Quarter Horses (2 to 3 yr; 401 to 432 kg BW) were used in a simple crossover design for a 49-d study to determine the effect of dietary starch levels on post-exercise glycogen replenishment. Horses were fed either high starch (HS) or low starch (LS) concentrates at 0.75% BW/d plus l.0% BW/d Coastal Bermudagrass hay for 14 d, and then worked to fatigue in a standardized exercise test (SET). After a 14-d washout period, horses were switched to the opposite diet for 14 d and then again performed the SET. The LS and HS concentrates were commercially available feeds. Total diets provided an average of 997.6 g of starch and 553.7 g of starch/d in the HS and LS diets, respectively. Throughout the trial, horses were lightly exercised for 30 min, 3 d/wk. The SET consisted of a 30-min warm-up period at a brisk trot in a panel exerciser, followed by 27 min of gradually ascending high-intensity work on a treadmill.

Skeletal muscle biopsies were taken from the biceps femoris at rest, immediately after the SET, and again at 6-, 24- and 48-h post-exercise. Samples were flash frozen in liquid nitrogen and stored at -80°C until analysis for later muscle glycogen concentration using a commercial kit. Venous blood samples were taken at rest, immediately post exercise and every 15 min for 3 h post-exercise. Blood samples were analyzed for lactate, glucose, total protein and Ca concentration. Data were analyzed using Proc Mixed (SAS) procedure with main effects of sample time, horse, period, trt and time x trt interaction.

Horses on the HS diet had a higher muscle glycogen concentration ($P < 0.05$) at 48 h post exercise than the LS horses (18.1 vs. 10.6 µg/mg wet wt). At 6 h, HS horses had a more rapid rate of repletion, as observed by the higher glycogen concentration ($P < 0.05$)

compared to the immediate post-exercise samples (15.4 vs. 7.9 μg/mg wet wt); whereas, the LS horses did not return to normal levels until 24 h post SET. Results indicated that horses on the LS diet, which is representative of low-starch feeding programs commonly observed in the industry, replenish their skeletal muscle glycogen slower than horses on the HS diet. Based on this study, performance horses undergoing multiple bouts of intense exercise may benefit physiologically from receiving diets that contain more than 553.7 g of starch/day.

ACKNOWLEDGEMENTS

NOMENCLATURE

bpm	Beats Per Minute
bw	body weight
d	day
DM	Dry Matter
hr	hour
HS	High Starch diet
LS	Low Starch diet
m	meters
min	minute
rpm	rotations per minute
trt	treatment
yr	year

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CHAPTER I

INTRODUCTION

The prevalence of multi-day competitive events throughout the performance horse industry has exacerbated the necessity for the horse to recover from intense exercise in a relatively short period of time. Recovery from fatigue is of particular interest to subsets of the industry where multiple bouts of intense, anaerobic exercise are asked for within a 24 or 48-hour period. Events such as reining, cutting, rodeo, jumping and working cow horse call for multiple preliminary runs before a final over the period of 1 to 2 d, which severely limits the amount of time for the horse to recover from one run to the next.

During such events horses are often asked to perform anaerobically for several seconds to several minutes, but often these brief moments of competitive work punctuate longer periods spent warming up or training. Horses may be over-exerted in the warm-up or preliminary runs so that they are unable to perform optimally in the final. The economic implications of premature fatigue and prolonged recovery are related to decreased performance during the later runs of multi-go events—where a loss of performance yields a loss of award money and may even increase the potential for fatigue-related injury.

A recent increase in the popularity of high fat, high fiber diets has further complicated this issue, particularly because anecdotal evidence has shown that horses on high fiber/low starch diets may fatigue at multi-day competitions faster than horses on moderate or high starch diets. This premature fatigue is likely due to the fact that horses on high fiber/low starch diets have a lower resting skeletal muscle glycogen concentration than horses on high starch diets (Kline and Albert, 1981). Arguably, an increased recovery

time also occurs because horses are less able to replenish their skeletal muscle glycogen stores before the next exercise bout.

Glycogen is the primary source of energy in skeletal muscles during exercise. Depletion of glycogen stores in the skeletal muscle is associated with fatigue in both human and equine athletes performing endurance and sprint-type exercise. Human athletes can be observed "hitting a wall" after long-term low-intensity exercise, which is associated with a near complete depletion of skeletal muscle glycogen (Jentjens and Jeukendrup, 2003). Horses that have been previously depleted of up to 60% of their skeletal muscle glycogen in a laboratory setting had a shorter time to fatigue than non-depleted horses (Davie et al., 1996).

The horse is 3-4 fold slower at replenishing skeletal muscle glycogen than the human (Waller and Lindinger, 2010). Whereas humans and rodents can replenish their glycogen stores in as little as 4-6 h (Jentjens and Jeukendrup, 2003), horses can require up to 48 h for complete replenishment (Lacombe et al., 2003). Researchers have successfully up-regulated human skeletal muscle glycogen repletion with nutritional intervention but have little success in significantly increasing the rate of repletion in the equine (Waller, 2010). Therefore, it is warranted to further explore other mechanisms to nutritionally up-regulate equine skeletal muscle glycogen replenishment to decrease recovery time and increase overall athletic performance.

The objectives of this study were to:

-Compare post-exercise glycogen concentration and other markers associated with fatigue in horses fed high starch or low starch diets, and

-Compare post-exercise skeletal muscle glycogen repletion patterns in horses fed a high or low starch diet.

Horses fed a high starch diet should have an increased rate of glycogen replenishment for several reasons. Among those explanations, is that a higher starch diet yields an increased availability of substrate (glucose) for early (insulin independent) glycogen synthesis. Horses on high starch diets versus high fat/fiber diets have also been shown to have a more rapid insulin response (Hoffman et al., 2003a); this is significant for up-regulation of later insulin-dependent glycogen replenishment.

CHAPTER II

LITERATURE REVIEW

Premature fatigue is a significant issue in the performance horse because it often leads to economic losses in prize money, training fees and possible injury. Fatigue was defined by Gibson and Edwards (1985) as the inability to maintain an activity at a prescribed intensity. Performance horses competing in high intensity events such as reining, cutting or jumping are most affected by fatigue, because horses are often warmed up for an extensive amount of time then asked for multiple bouts of intense exercise. There are many causes of fatigue, including overheating, dehydration, lactate buildup and limited energetic substrate availability (Lacombe et al., 2003).

Dietary starch concentration may affect how quickly performance horses recover from fatigue by affecting the rate of skeletal muscle glycogen replenishment. Glycogen depletion can be a cause of acute fatigue, particularly the highly anaerobic exercise which is common in equine events (Waller and Lindinger, 2010). Glycogen replenishment is a significant part of recovery from fatiguing exercise, and is dependent on starch digestion and metabolism. Both glycogen depletion and repletion rely on endocrine coordination. Nutritional interventions are sometimes used to increase the rate of glycogen replenishment in humans and may have applications in the equine (Lacombe et al., 2003).

Glycogen Depleting Exercise

Glycogen depletion in skeletal muscle has been associated with fatigue in both horses and humans (Lacombe et al., 1999; Davie, 1996; and Jentjens and Jeukendrup, 2003). In most glycogen-related equine studies, researchers have been unsuccessful in

significantly depleting glycogen stores in skeletal muscle, because glycogen depletion

appears to occur after several days of repeated anaerobic exercise. Glycogen depletion

occurs when the skeletal muscle cell cleaves glucose units off of a larger glycogen

molecule during aerobic or anaerobic exercise. The rate of glycogen utilization is

dependent on type of activity, diet and intensity of the activity (Julen et al., 1995; Hodgson

et al., 1984; McCutcheon, 1999; Schuback and Essen-Gustavsson, 1998).

Hodgson et al. (1984) examined glycogen depletion patterns in horses performing

endurance exercise. Type I muscle fibers were recruited first and stores of glycogen in the

fibers were depleted before the recruitment and subsequent depletion of Type II a and b

fibers. This depletion pattern was not dependent on activity; however, the degree of type II

fiber recruitment and glycogen depletion is a reflection of the intensity of the activity.

Essentially, horses performing low intensity, aerobic exercise displayed the same pattern of

glycogen depletion as horses performing high intensity exercise, but horses performing

high intensity exercise had a much greater degree of depletion in type II fibers.

Lacombe et al. (1999) demonstrated significant skeletal muscle glycogen depletion,

achieved by 3 d of repeated sprints (calculated to be 120% VO_{2MAX}), which caused a

decrease in anaerobic performance. The horses in this study, which had a 55% reduction in

skeletal muscle glycogen concentration, had a shorter time to fatigue compared to horses

that were not worked during the three d preceding the exercise test. This is significant

because these horses were rested between exercise bouts, which allowed for superficial

recovery (lactate clearance, temperature regulation), but not glycogen replenishment. The

fact that the previously-worked horses fatigued sooner than their rested counterparts, showed the relationship of energy substrate availability to anaerobic performance.

In contrast, Davie et al. (1996) found that time to exhaustion was not affected in horses depleted of 22% of their skeletal muscle glycogen. However, the work of Topliff et al. (1985) showed that a 41% decrease in muscle glycogen concentration (achieved by five d of glycogen depleting exercise) led to decreased work output when horses were asked to perform anaerobically by pulling a weighted sled.

Although there are no current studies that have examined the magnitude of glycogen depletion achieved by performance horses in a competitive setting, the fact that horses are worked at high intensities during competitive rides and worked more aerobically during training rides with minimal recovery, makes multi-day studies including those by Lacombe et al. (1999) and Topliff et al. (1983) more applicable to the performance horse industry. Many performance events require 2-3 bouts of highly anaerobic work within a 24-48 h period. As a result, based on the magnitude of glycogen depletion determined by studies such as Lacombe et al. (1999), it may be useful to explore ways to mitigate this facet of fatigue.

Starch Metabolism in the Equine

The horse is a hind-gut fermenting ungulate that evolved to extract maximal energy from structural carbohydrates found in grasses. Equines' gastro-intestinal physiology is not designed to extract soluble carbohydrate in the small intestine like the majority of non-ruminants, such as humans (Hoffman, 2009). However, in the performance horse, soluble carbohydrates are supplemented to increase caloric intake to compensate for energy

expended during exercise. The lower capacity for starch digestion is due to a relatively small and inactive intestinal enzyme population and a rapid rate of passage in the foregut (stomach and small intestine)(Kienzle et al., 1997).

Pre-cecal digestibility of concentrate depends on the material and the degree of processing that the substance undergoes before it is fed (Kienzle et al.,1997). When excess starch is fed at one time or an excess of soluble carbohydrate is fed in an indigestible form, then the majority of that starch bypasses hydrolysis in the foregut to be broken down by cecal bacteria (Clarke et al., 1990). If greater than 2-4 g starch/kg bw is fed in one meal (Kienzle et al., 1997), the "bypass starch" can cause systemic acidosis and bacterial death in the cecum, which can lead to metabolic diseases including laminitis (Hoffman, 2009).

When a horse consumes a concentrate meal, the bolus first travels from mouth to stomach. In the stomach, a minimal amount of carbohydrate hydrolysis occurs; lactic acid bacteria convert large starch molecules to smaller starch molecules and lactate. In the small intestine, pancreatic peptidases, lipases and amylase are secreted. The small intestine is also the primary location of starch absorption. The enzyme SGLT1 is present in intestinal epithelium and shuttles carbohydrate monomers and dimers from the lumen of the small intestine to the blood supply. These glucose molecules are then transported to the liver where they will be stored as glycogen, circulated to increase blood glucose levels or converted to triglycerides and stored as adipose tissue (Keinzle et al., 1997).

Glycogen is synthesized from free glucose by glycogen synthase around a core protein called glycogenin. Glycogen synthase is activated by a series of reactions as a response to insulin signaling. Phosphorylated glucose residues are linearly attached by α-

1,4 bonds, but branch (via an α-1,6 bond) every 10-14 residues to maximize the solubility and eventual energy availability of the glycogen molecule (Brjoer et al., 2002). These long chains of glucose units are stored in the muscle cell to be broken down as an energy source.

Endocrine Control of Starch Metabolism

When blood glucose concentration increases, such as after a meal, the pancreas releases insulin. Insulin causes the glucose-transporting enzyme GLUT4 to translocate to the membrane of muscle cells to facilitate the diffusion of glucose from the blood into the muscle cell (Waller et al., 2011). Insulin is also responsible for increasing the rate of glycogen synthesis by causing the phosphorylation of glycogen synthase, the rate limiting enzyme in glycogen synthesis (Pratt et al., 2007).

Throughout exercise, catecholamines such as epinephrine and norepinephrine are released to optimize systemic availability of energy substrate to power skeletal and cardiac muscle contraction (Hyppa, 2005). These hormones facilitate glycogen hydrolysis, mobilization of adipose tissue, and inhibit the release of insulin to maximize glucose availability for ATP production. According to Poso and Hyppa (1999) and Stull and Rodieck (1995), this systemic mobilization of glucose during exercise causes insulin levels to increase approximately one h after exercise as part of the recovery process.

The majority of insulin-centric studies have focused on starch-rich diets as a cause of insulin resistance in both humans and horses (Secombe and Lester, 2012, Hoffman, 2009, Kronfeld et al., 2005 and Kronfeld and Harris, 2003). Horses and humans adapted to a high starch/sugar diet, without exercise, have been shown to require a greater

concentration of insulin for blood glucose clearance (Stewart-Hunt et al., 2010, Secombe and Lester, 2012). However, according to Goodyear and Kahn (1998) exercise mitigates the insulin resistance associated with excess soluble carbohydrate consumption in the human. The same was shown to be true by Stewart-Hunt et al. (2010) in horses adapted to a high starch diet with regular exercise.

Hoffman et al. (2003a) demonstrated that mares adapted to a high starch/sugar diet have a more rapid insulin release and subsequent systemic response to insulin, in the form of blood glucose clearance, compared to horses on a isocaloric high fat/fiber, low starch diet. Therefore, horses adapted to a higher starch/sugar diet are better equipped to respond to fluctuations in blood glucose, such as the increase blood glucose that occurs in exercise. This more rapid insulin response may also result in a faster up-regulation of glycogen synthesis in horses adapted to a high starch diet.

Endocrine Response to Exercise

Throughout high intensity exercise, the horse needs to increase its metabolic rate up to 60 times, compared to rest (Hyppa, 2005). To maintain the high level of metabolic activity associated with multiple and rapid skeletal muscle contractions, energy stores are mobilized in the form of glucose. This glucose supply is primarily from skeletal muscle glycogen, which is cleaved and released in response to glucagon, adrenaline and cortisol. Although glucagon is largely released in response to a transient, longer term drops in blood glucose, adrenaline and cortisol both elicit a more immediate response to increase blood glucose in "fight" or "flight" situations (Brooks et al., 1988).

Catecholamines released in response to exercise increase blood glucose concentration by mobilizing energy stores (glycogen depletion) and by inhibiting insulin release (Hyppa, 2005). However, during recovery, approximately one h after the completion of exercise, insulin levels increase to greater than the resting levels (Hyppa, 2005 and Lacombe et al., 2004). The reason for this spike is to clear excess glucose from the blood and to stimulate glycogen replenishment.

Insulin controls glycogen replenishment in a variety of ways, most importantly, the activation of intracellular glycogen-synthesizing hormones and facilitating glucose diffusion into the cell from the blood (Dent et al., 1990). The importance of insulin in the replenishment of glycogen in the skeletal muscle has led both human and equine nutritionists to elicit a more dramatic and earlier insulin spike with post exercise meals that are high in glucose and amino acids, such as leucine (Jentjens and Jeukendrup, 2003, and Poso and Hyppa, 1999).

Human Post-Exercise Nutritional Intervention

In human studies, researchers have successfully increased the rate of glycogen re-synthesis by administering a simple carbohydrate supplement post exercise. According to several researchers, it is possible to increase the rate of post-exercise glycogen synthesis almost two-fold to 50 mmol/kg dw/h (Jentjens and Jeukendrup, 2003) compared to a non-supplemented rate of 24 mmol/kg dw/h. Blom (1989) successfully increased skeletal muscle glycogen storage rate by 150% (9.0-24.8 mmol/kg dw/hour) by administering 0.18 to .035 g carbohydrate/kg/h.

Research conducted by Ivy et al. (1988) showed glycogen is synthesized at a 45% slower rate when glucose is administered 2 h after exercise compared to administration immediately after exercise. Conversely, Parikin et al. (1997) found no discernible difference between glycogen synthesis rates in individuals given a carbohydrate meal immediately after exercise and 3 h post exercise. Blom et al. (1989) found a maximal rate of repletion by administering carbohydrate meals every thirty minutes post exercise.

Van Loon et al. (2000) and Zawadzki et al. (1992) examined the effect of amino acid supplementation on glycogen repletion. Specifically, whey protein given in combination with soluble carbohydrates was shown to increase glycogen synthesis by eliciting an increased insulin response, which in turn increased the rate of glycogen synthesis by increasing cellular glucose uptake and activating glycogen synthase (Zawadzki et al., 1992). Other researchers have found that carbohydrate and whey protein plus leucine or phenylalanine also increases rate of glycogen synthesis (Ivy et al., 2002).

Fats, both long and short chain, are supplemented in different ways to increase glycogen synthesis and/or availability. In human and rodent studies, subjects adapted to a high-fat diet showed a decrease in the resting glycogen concentration (Conlee et al. 1990). Other studies in humans show that supplemented fat in non-adapted subjects showed an initially slower rate of glycogen re-synthesis compared with a lower fat, high carbohydrate diet (Decombaz et al, 2001). However, Burke et al. (2000) found that fat adaptation is beneficial for cycling performance because of a glycogen sparing effect. In fat-adapted cyclists, muscle cells preferentially use triglycerides during aerobic metabolism, "sparing" glycogen stores and increasing time to fatigue (Jentjens and Jeukendrup, 2003).

Equine Post-Exercise Nutritional Intervention

Carbohydrate supplementation is one of the most popular post exercise nutritional strategies to increase the rate of glycogen repletion in equine skeletal muscle. This popularity is likely due to the widely held belief that glycogen re-synthesis is so slow in the horse because of a lack of available glucose substrate (Lacombe, 2001). However, researchers have been unsuccessful at increasing the rate of glycogen synthesis by supplementing oral or injected glucose (Waller, 2010). Several studies showed trends toward an increased rate of glycogen repletion with glucose supplementation, but none of these results were found to be statistically significant (Davie et al., 1996).

Investigators have attempted to increase the rate of glycogen repletion in the post-exercise horse by adding amino acids and/or whey protein to carbohydrate supplements. Poso and Hyppa (1999) found no improvement in the rate of glycogen repletion with horses supplemented leucine and a simple carbohydrate. Miller-Graber et al. (1991) recommend strongly against excess protein supplementation for the horse, as it is associated with decreased performance due to an increased urea load and acidosis. Reportedly, horses are not physiologically equipped to utilize protein as well as humans as an energy substrate.

Horses derive approximately 30-40% of their resting energy from volatile fatty acids (VFAs) absorbed in the large intestine after fermentation has occurred in the cecum (Waller, 2010). The most prevalent of the VFAs absorbed in the hind-gut is acetate, the shortest chain fatty acid, and a precursor to Acetyl CoA (Waller 2009b). There are two

studies to date that have attempted VFA supplementation post exercise to increase the rate of glycogen repletion in equine skeletal muscle.

Poso and Hyppa (1990) supplemented propionate through a nasogastric tube immediately post exercise. Although no significant changes in the rate of glycogen synthesis were found, researchers may not have supplemented enough propionate to elicit the endocrine response necessary for increasing the rate of glycogen synthesis.

Waller et al. (2009b) supplemented acetate with a typical hay and grain meal immediately post exercise. Acetate supplement significantly increased glycogen synthesis at four hours post exercise; however, there was not a significant treatment effect at twenty-four hours post exercise. This suggests that acetate was utilized quickly as a substrate for glycogen synthesis, and is a potential alternative to glucose for supplementation in the horse. However, Waller et al. noted that the acetate treatment group was less interested in their hay/grain meal and showed signs of dehydration, all of which could have affected glycogen synthesis in skeletal muscle.

It is hypothesized that one of the primary reasons why equines are slow at replenishing skeletal muscle glycogen stores is post-exercise dehydration. Glycogen synthesis requires adequate intracellular water availability and balance of intra- and extra-cellular electrolytes because glycogen is stored in the hydrated form. Exercise causes a net loss of water and electrolytes (via sweat and respiration). Waller et al. (2009a) successfully increased the rate of post-exercise glycogen replenishment by administering water and an electrolyte solution to six Standardbred horses after prolonged, moderate intensity

exercise. Although this may not be a nutritional strategy for increasing the rate of glycogen replenishment, it is notable for the purpose of controlling similar studies.

Equine Dietary Nutritional Intervention

"Glycogen loading" refers to a dietary intervention that increases resting glycogen concentration prior to exercise. Kline and Albert (1981) explored the effect of dietary carbohydrate on skeletal muscle glycogen "loading" in Standardbred horses. They found that horses fed a high carbohydrate diet for three days prior to an exercise test had a significantly higher resting glycogen concentration than horses on high fat and protein diets. This project was significant because it was the first to show that diet affects markers associated with fatigue in horses.

Topliff et al. (1983) examined the effect of diet on glycogen concentration and exercise tolerance. Horses fed a diet high in soluble carbohydrates had roughly 36% higher initial glycogen concentration in skeletal muscle compared to horses on a high fat, high protein diet. This work expanded on the work of Kline and Albert (1981) by increasing the length of time that horses were on the experimental diets. The fact that both investigators had similar results with differing adaptation periods shows that horses display a significant glycogen response after only a few days.

Pagan et al. (1987) compared the effects of three different diets on long, slow or intense, sprint exercise performance. Three Standardbred horses were fed a high starch, a high protein and a high fat diet for one-month periods. Each horse performed a low intensity endurance-type test during week three and a high intensity sprint test during week 4 of each dietary period. Horses fed the high starch diet had a higher resting and exercising

glycogen concentration than horses fed the high fat or high protein diet. Essentially, diet significantly affects markers of fatigue in performance horses.

Equine researchers have closely examined fat as an alternative energy source to carbohydrates, as fat is not associated with metabolic issues observed in horses fed simple carbohydrates (Kronfeld, 2003). However, a "high fat" diet in horses is much lower than what is considered a "high fat" diet in humans or mice (Waller, 2010). One of the strategies utilized when supplementing fat in the equine is to feed a high fat and high carbohydrate diet. Here, the fat creates a "glycogen sparing" effect—decreasing the amount of glycogen utilized during exercise especially when compared to glycogen utilization of horses on a high fiber diet (Topliff et al. 1985). It has also been shown that dietary fat supplementation may be associated with an increase in resting glycogen concentrations (Lacombe et al. 2003).

In horses adapted to a high fat diet over a period of 28 d, glycogen utilization during low intensity activity is less than in non-fat adapted horses (Julen et al., 1995). This is because type I skeletal muscle fibers preferentially use fat as an energy source at low intensities, which leaves more glycogen available for use during high intensity exercise. In the non-adapted horses, fat is a less available energy source so glycogen is utilized in both low and high intensity work. The fact that fat in the diet has a glycogen sparing effect, has changed the way performance horses are fed.

Conclusion

Glycogen depletion and subsequent replenishment are significant aspects of fatigue and recovery from fatigue. In the performance horse industry the standards of performance

and the standards of excellence in high intensity events is ever increasing. However, the trend in horse feeding has been toward lower starch feeds. There is currently no study on record that explores the correlation between dietary starch and recovery from exercise, measured by glycogen replenishment. Investigators have been largely unsuccessful in attempts to increase the rate of glycogen replenishment in horses with post-exercise meals. It appears that an increase in dietary starch in the daily diet should increase the rate of glycogen synthesis in skeletal muscle by increasing glucose availability and the magnitude of the post exercise insulin response.

CHAPTER III

MATERIALS AND METHODS

Animal Management

American Quarter Horse mares ($n = 3$) and geldings ($n = 1$), 2 to 3 yr old (average

BW = 401kg), owned by the Texas A&M University Department of Animal Science were

used in a two-way crossover design for a 52-d study. Horses were selected based on

temperament, and lack of apparent metabolic disease or unsoundness. The horses were

vaccinated and dewormed prior to the study according to a regular schedule maintained by

the Texas A&M Horse Center. Horses were housed in four paddocks at the Texas A&M

University Horse Center and managed in accordance with the guidelines established by the

Institutional Animal Care and Use Committee.

The study consisted of two 14-d experimental periods during which horses were

fed one of two concentrates, three 7-d transition periods during which horses were

gradually switched to a new concentrate and one 7-d washout period wherein each horse

was fed the same concentrate. Horses were fed the same batch of Coastal Bermudagrass

(*Cynodon dactylon*) hay throughout the experiment. The concentrate, particularly the

starch concentration in the concentrate, was the only variable that changed from treatment

to treatment. Horses were weighed weekly and their rations were adjusted appropriately.

Dietary Treatment

Horses were fed one of two commercial pelleted concentrates, high starch (HS) or

low starch (LS). The concentrates were fed at 0.75% BW/d, ensuring each horse received

an average of 3.02 kg concentrate/day. Horses also received 1% BW (as fed) of Coastal

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Bermudagrass hay daily. Daily hay and concentrate rations were divided equally into two meals, fed at 0730h and 1630h daily. Hay and concentrate were offered to each horse in individual elevated feeders. Horses had *ad libitum* access to water throughout the study. Refusals at the time of the next feeding were collected and weighed. Total analysis of each concentrate and hay are presented in tables 1, 2 and 3.

Table 1. Starch Concentration of Dietary Components (100%DM)

Component	Starch Concentration, %
High Starch Concentrate[a]	29.95
Low Starch Concentrate[b]	15.20
Coastal Bermudagrass Hay	2.40

[a] 14% CP pelleted concentrate (Producers Cooperative Association, Bryan, TX)
[b] Commercial Safe Choice Special Care (Cargill Inc.)

The HS feed was used as a washout feed for 7 d prior to the first experimental diet period and during the 7-d washout period. The HS feed was used because horses were on a very similar diet before the start of the study.

Prior to each 14-d experimental diet period, horses were gradually switched to the new diet over a period of 7 d. The first two d (4 feedings) horses were fed 25% of the new diet (i.e., LS concentrate) and 75% of the previous diet (i.e., washout concentrate). The next three d (6 feedings) were presented at 50% new diet and 50% previous. The final two d (4 feedings) contained 75% new diet and 25% of the previous diet.

Table 2. Total Analysis of Coastal Bermudagrass Hay (100% DM)

	Constituent
Moisture, %	7.50
Dry Matter, %	92.50
Crude Protein, %	16.60
Acid Detergent Fiber, % (ADF)	32.70
Neutral Detergent Fiber, % (NDF)	62.20
Water Soluble Carbohydrate, %	8.20
Calcium, %	0.55
Phosphorous, %	0.24
Magnesium, %	0.24
Potassium. %	1.54

Table 3. Analysis of Dietary Treatments (100% DM)

	Treatment	
	HS[a]	LS[b]
Moisture, %	9.70	10.05
Dry Matter, %	90.30	89.95
Crude Protein, %	17.05	17.35
Acid Detergent Fiber, % (ADF)	16.25	23.95
Neutral Detergent Fiber, % (NDF)	28.20	39.25
Water Soluble Carbohydrate, %	6.40	7.75
Calcium, %	0.95	0.99
Phosphorous, %	0.69	0.74
Magnesium, %	0.26	0.34
Potassium	1.10	1.32
Sodium	0.27	0.54

[a] 14% CP pelleted concentrate (Producers Cooperative Association, Bryan, TX)
[b] Commercial Safe Choice Special Care (Cargill Inc.)

Exercise Protocol

Prior to the trial, all four horses had previously been used in Texas A&M University, Department of Animal Science horse training labs. Horses were worked throughout the trial at a walk and trot in a panel exerciser (Freestyle Equine Equipment, Unadilla, GA) for 30 minutes, 3 d/wk to maintain, but not increase fitness. Horses traveled

an average of 5.8 km per exercise session at an average speed of 4.1 m/s. When horses

were not being worked they were turned out in individual pens. Horses were rested for 3 d

prior to the exercise tests. After exercise tests, horses were rested for 7 d before resuming

the light exercise schedule.

After each 14-d experimental diet period each horse performed a strenuous

standard exercise test. Horses were first worked for 30 min at a brisk trot (4.1 m/sec) in a

panel exerciser (Free Style Equine Equipment) then moved to a high-speed treadmill (Sato,

Upsala, Sweden) to be worked to fatigue. A horse was considered fatigued when the heart

rate remained above 180 bpm for more than 30 s, the horse completed the full exercise test,

or the horse could no longer keep pace with the treadmill. Heart rates were monitored

throughout the test using a heart rate monitor (Polar Electro, Lake Success, NY). Intensity

of exercise was incrementally increased with both speed and incline of the treadmill.

Table 4. Standard Exercise Test

Time (min)	Speed (m/s)	Incline
30[a]	4.1	0.0°
2[b]	1.9	0.0°
5[b]	3.0	0.0°
5[b]	3.0	2.1°
5[b]	5.6	2.1°
5[b]	5.6	2.8°
5[b]	6.5	2.8°

[a] Performed in panel exerciser (Freestyle Equipment)
[b] Performed on high speed treadmill (Sato)

Sample Collection

Muscle

Muscle samples were collected immediately before the exercise test, immediately after the exercise test, and at 6, 24 and 48 h post exercise from the biceps femoris. Samples were taken from the biceps femoris at a consistent depth of approximately 3 cm. Animals were restrained in stocks for the procedure using a lip twitch as needed.

Immediately prior to the muscle biopsy procedure, area over the biceps femoris muscles was clipped and disinfected aseptically using betadine scrub (providine-iodine 5%), chlorohexadine (2% chlorohexadine gluconate, Liberty, MO), and a 70% alcohol solution (70% isopropyl alcohol). The skin immediately superficial to the intended muscle sample site was locally anesthetized using 2.5 ml lidocaine (2% lidocaine hydrochloride, VetOne, Biose, ID), administered subcutaneously using a 25G needle (Kendall Co., Mansfield, MA). The lidocaine was allowed 1-2 min to take effect before proceeding.

The biopsy procedure was adapted from protocol described by Tarnopolsky et al. (2011) and Snow and Guy (1976). A 5 mm Bergstrom (Stille, Stockholm, Sweden) biopsy needle was fit with 20 cm plastic tubing, attached to a 60 ml syringe (Kendall Co.). A small amount of sterile lubricant (Priority Care, Elgin, IL) was added to the internal sleeve of the biopsy needle, prior to the procedure, to create a tight seal when both the internal and external sleeve was used.

A 5-6 mm stab incision was made using a 5 mm scalpel through the center of the previously anesthetized skin and fascia. The biopsy needle was inserted 3-4 cm deep through the incision. The inner cutting sleeve was retracted from the outer sleeve and

suction was applied to draw muscle tissue into the biopsy needle. The inner sleeve was used to cut a sample of muscle by pressing it into the outer needle with a twisting motion. The entire needle was rotated 90° to the left to take a sample then 180° to the right to take a final sample.

After this, the entire apparatus was withdrawn from the horse and ice and pressure applied to the area of the incision. When the incision was no longer bleeding, super glue (FutureGlue, Ranch Cucamonga, CA) was applied to keep the incision closed. No sutures were necessary to close any of the incisions. To prevent infection, a topical antibacterial ointment (Dermagel, Maximilian Zenho & Co., Brussels, Belgium) was applied after the super glue dried. Horses were monitored for lameness and infection over the next several days.

The muscle sample was immediately frozen in liquid nitrogen by fully immersing the sample, wrapped in foil. After immersion for approximately 5 min, the sample was transferred to a 2 ml cryogenic vial (VWR, West Chester, PA) for storage at -80°C until analysis.

Blood

Blood samples were collected via repeated jugular venipuncture at the time of each biopsy and every 15 min for three h immediately post exercise into sodium-heparin added sterile blood collection tubes (Kendall Co.). Blood samples were immediately placed on ice in an insulated cooler and centrifuged within 15 min of collection at 2500 rpm for 12 min. Serum was then pipetted into labeled 1.5 ml micro-centrifuge tubes (VWR) and frozen at -20°C for later analysis

Sample Analysis

Feed Analysis

Grab samples of both diets were taken from each 22.7 kg bag used during both 14-d experimental periods. These samples were compiled and frozen and a sub sample shipped for analysis. A representative hay sample also was taken from several different bales using a hay bale sampler. The four pelleted grain samples and one hay sample were analyzed for % DM, CP, ADF, NDF, starch, water-soluble carbohydrates, Ca, Mg, Na and P by EquiAnalytical Laboratories (Ithaca, NY).

Glycogen

Glycogen concentration of muscle samples was analyzed using a commercial ELISA kit (Biovision Inc., Milpitas, CA). Frozen muscle samples were weighed and a 10-12 mg sub-sample removed for analysis. The thawed sub-sample was diluted in distilled water and homogenized. The samples were then boiled for 5 min and centrifuged at 13000 rpm. The supernatant was then further diluted (2:1) in the ELISA wells using the kit-provided buffer solution. The provided development enzymes and colorimetric solution was added to this and the plate allowed to incubate for 30 minutes in the well reader (Beckman Coulter, Pasadena, CA). The readings were internally validated for precision using a standard value (2 mg/mL). The well reader was calibrated within one year of the assay to be accurate up to 1 absorbance unit.

The kit measured NADPH using absorbance at 570 nm. This value was used to determine glucose concentration of five standard glycogen solutions to create a standard

curve. The glycogen concentration (wet wt) of each muscle sample was calculated from the regression of the standard curve.

Glucose, Lactate, Ca and Total Protein

Serum samples collected in the three h immediately post-exercise were analyzed by the Texas A&M University College of Veterinary Medicine internal diagnostic lab. Samples were analyzed for glucose, lactic acid, total protein, Ca and Mg concentrations.

Statistical Analysis

Data were analyzed utilizing a multivariate analysis of variance (MANOVA) Glycogen data was analyzed using the PROC Mixed program in SAS. Model included effects of horse, period, sample time, treatment and time x treatment interaction. Horse nested in treatment for repeated measure, time point 0 was removed because of presumed lack of treatment effect. Differences were considered significant when $P \leq 0.05$.

CHAPTER IV

RESULTS

Dietary Starch Intake

Daily dietary starch consumption was determined by multiplying the concentration

of starch in both the hay and concentrate (% as fed) by the average amount of feed fed to

each animal per day (0.75% BW concentrate and 1% in hay). This number was then

averaged to obtain a representative number (401kg), as horses did not significantly gain or

lose weight throughout the project, and intakes did not differ from horse to horse. There

were no refusals from any part of the project. The results of the analysis (Table 5) show

that the HS diet had a higher starch concentration than the LS.

Table 5: Average Dietary Starch Intake/d, Including Concentrate and Hay, as fed

Diet	Starch Intake (g/day)
Low Starch	553.7
High Starch	997.6

Heart Rate

Heart rates (HR) were recorded every 5 min throughout the exercise test (Figure 1).

There was a significant effect of time on HR ($P < 0.05$) but not treatment ($P = 0.232$). As a

whole, under both treatments, HR increased as a function of time, which is also arguably a

function of the exercise intensity.

Figure 1: The effect of diet on heart rate during exercise (Mean ± SE[1])

Mean Heart Rates During Exercise± SE[1]
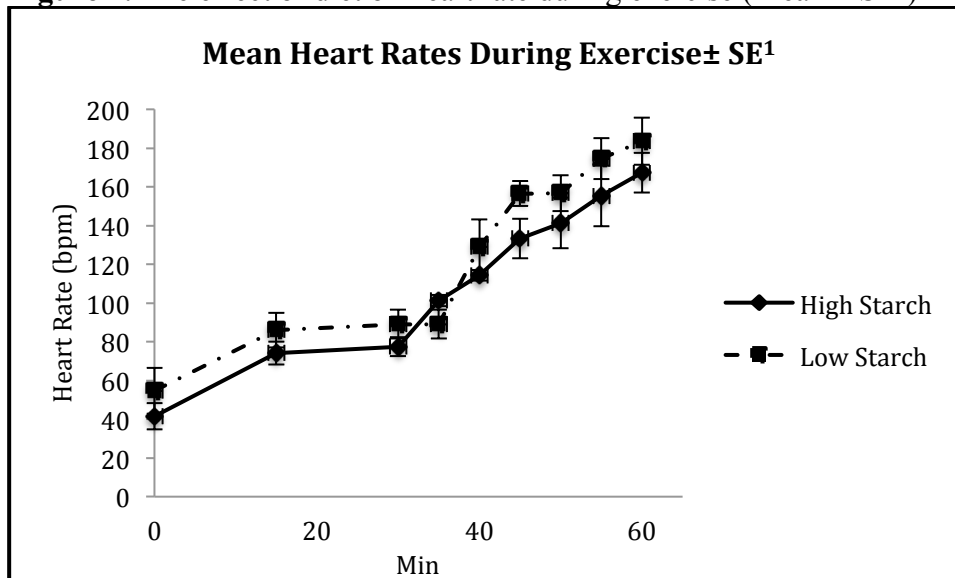
[1] SE = Standard Error

Skeletal Muscle Glycogen Concentration

The average skeletal muscle glycogen concentration in muscle samples taken at rest, immediately post exercise, 6, 24 and 48 h post exercise is presented in Figure 2 and Table 6. The concentration of glycogen at rest and immediately after exercise is not different between horses on HS and LS diets. At the 6 h sample, there is a significant effect of sampling time on glycogen samples taken from horses on a HS diet ($P<0.05$), but not the LS. At the 24 h sample the HS concentration remained the same as the 6 h sample, but there was an increase in concentration noted on the LS horses ($P<0.05$). There was a treatment effect in the concentrations taken 48 h after exercise ($P<0.05$) with the concentration of HS samples remaining at resting concentration and the LS horses lower in glycogen concentration.

Table 6: Mean Skeletal Muscle Glycogen Concentration (µg/mg wet wt)

| Time of sample	High Starch Concentration	SE[1]	Low Starch Concentration	SE
Rest	19.85[a]	5.10	18.74[a]	1.97
Post exercise	7.91[b]	2.12	9.66[b]	1.55
6 h	15.44[a]	3.98	11.03[b]	2.99
24 h	17.39[a]	1.81	16.57[a]	1.10
48 h	18.14[a] [*]	1.06	10.62[b]	2.30

[1] SE = Standard error of the mean
[a,b]Values in the same column not sharing the same superscript differ (P< 0.05)
*Values in the same row differ (P<0.05).

Figure 2: The effect of diet and time (h) on glycogen concentration (µg/mL) (Mean ± SE[1])
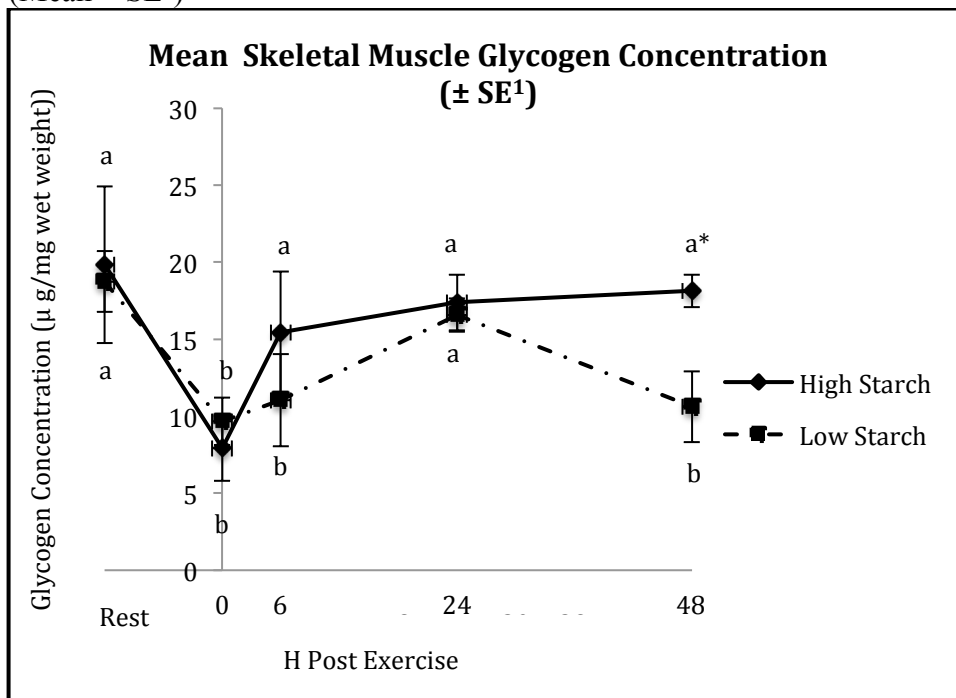
[1] SE = Standard Error
[a,b]Values in the same treatment not sharing the same superscript differ (P< 0.05)
*Values at the same sample time differ (P<0.05).

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To cancel out some of the variation due to individual resting glycogen concentration, the relative concentration of skeletal muscle glycogen was determined (Table 7 and Figure 3); by determining the ratio of each time point concentration to the pre-exercise concentration. As a trend, these ratios show that horses on the HS diet used more glycogen during the SET, but tended to replenish their stores at a faster rate. The HS horses had a greater glycogen concentration than the LS at the 48 h post exercise time point.

Table 7: Relative Skeletal Muscle Glycogen, Shown as a Ratio to Resting Concentration. (Mean ± SE[1])

	High Starch		Low Starch	
Time of Sample	Ratio	SE[1]	Ratio	SE[1]
Post exercise	0.405^a	0.0819	$.5225^a$	.115
6 h	0.8075^b	0.40877	0.600^a	0.36887
24 h	1.0975^b	.6362	$.9025^b$	.1707
48 h	1.100^b*	0.49	0.59^a*	.315

[1] SE = Standard Error
[a,b]Values in the same column not sharing the same superscript differ (P< 0.05)
*Values in the same row differ (P<0.05).

Figure 3: Ratio of glycogen concentration after exercise to resting concentration on High and Low starch diets (Mean ±nd L.
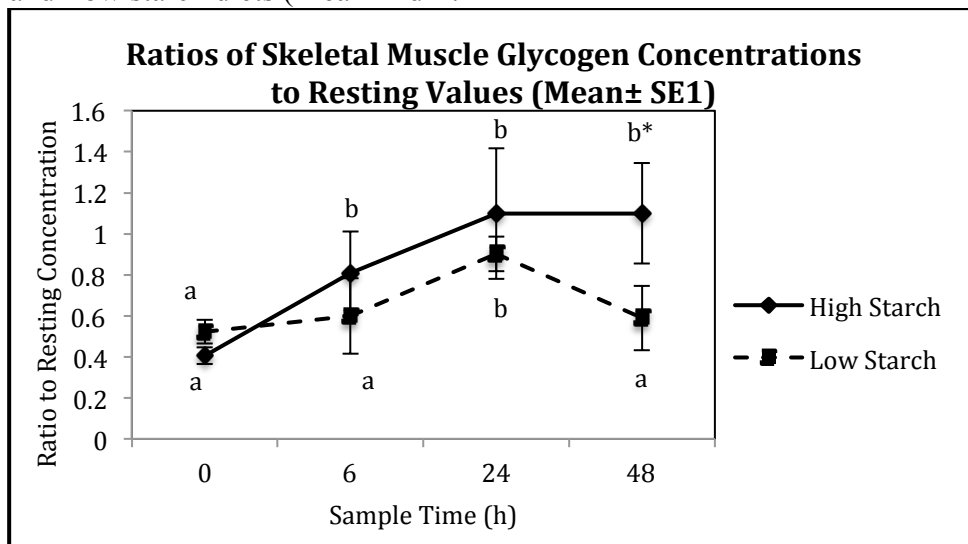
Ratios of Skeletal Muscle Glycogen Concentrations to Resting Values (Mean± SE1)

[1] SE = Standard Error
[a,b] Values in the same treatment not sharing the same superscript differ (P< 0.05)
*Values in the same time point differ (P<0.05).

Glucose and Lactate Concentration

There was an effect of time (P<0.05), but not treatment (P=0.303) on both lactate (Figure 4) and glucose (Figure 5). Both metabolites peaked in concentration immediately post exercise and were back to resting levels by 3 h post exercise. Horses on the HS diet had a slightly, but consistently higher concentration of glucose than horses on the LS diet.

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Figure 4: The effect of diet on lactate concentration taken every 15 min after exercise

Figure 5: The effect of diet on glucose concentration taken every 15 minutes after exercise. (Mean $\pm$ SE[1])
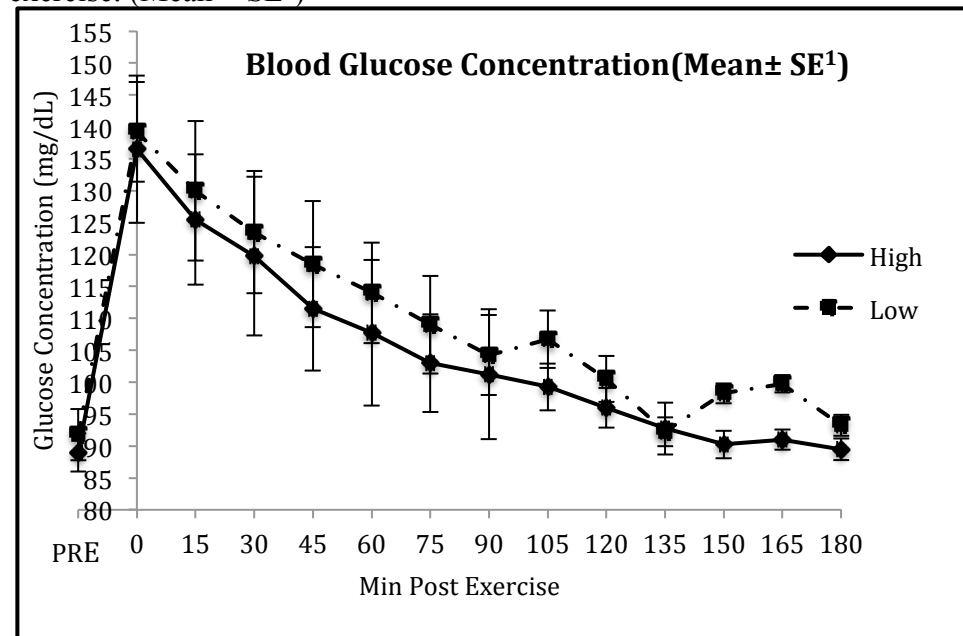
Total Protein and Ca

The total plasma protein (Figure 6) and Ca (Figure 7) concentrations did not vary as much as lactate and glucose, but there was a time effect on concentration. The total plasma protein concentration served as a marker of dehydration and peaked immediately after exercise ($P<0.05$) then varied throughout recovery. The Ca concentration was highest at rest and significantly decreased ($P<0.05$) immediately after exercise and gradually increased to resting level throughout the three h recovery period.

Figure 6: Total **s**erum **p**rotein **c**oncentration **c**ompared to **t**ime and **d**iet in **s**amples **t**aken **e**very 15 min for 2 h **a**fter **e**xercise (Mean ± SE)
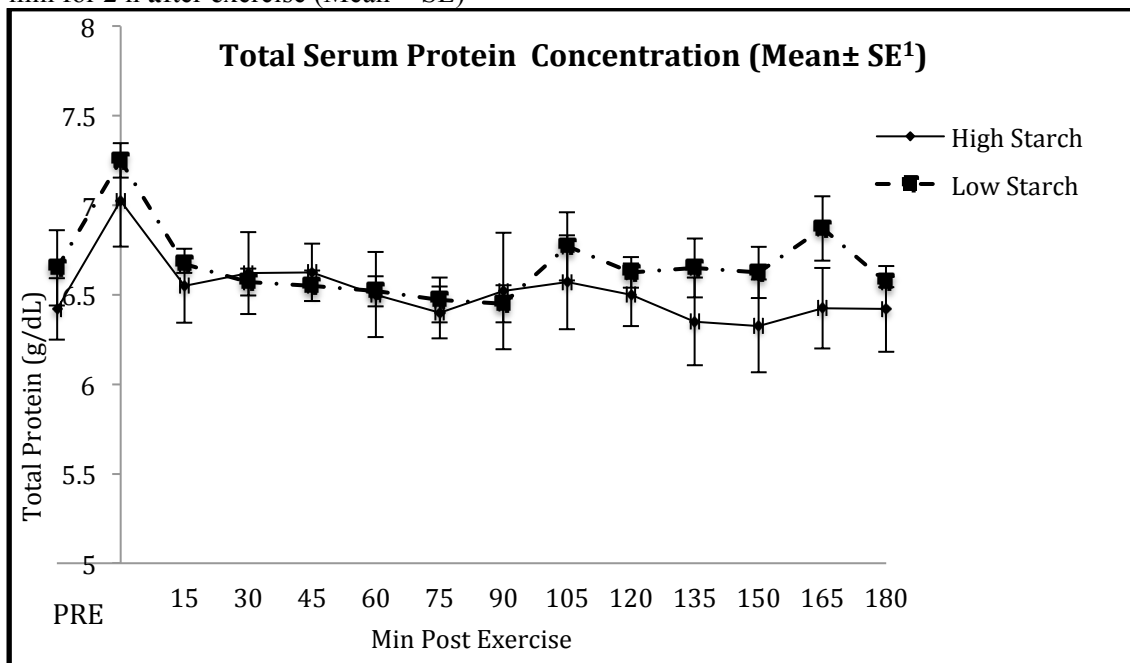
[1] SE = Standard Error

Figure 7: Ca concentration compared to diet and time in serum samples taken every 15 min for 3 hr after exercise (Mean ± SE)
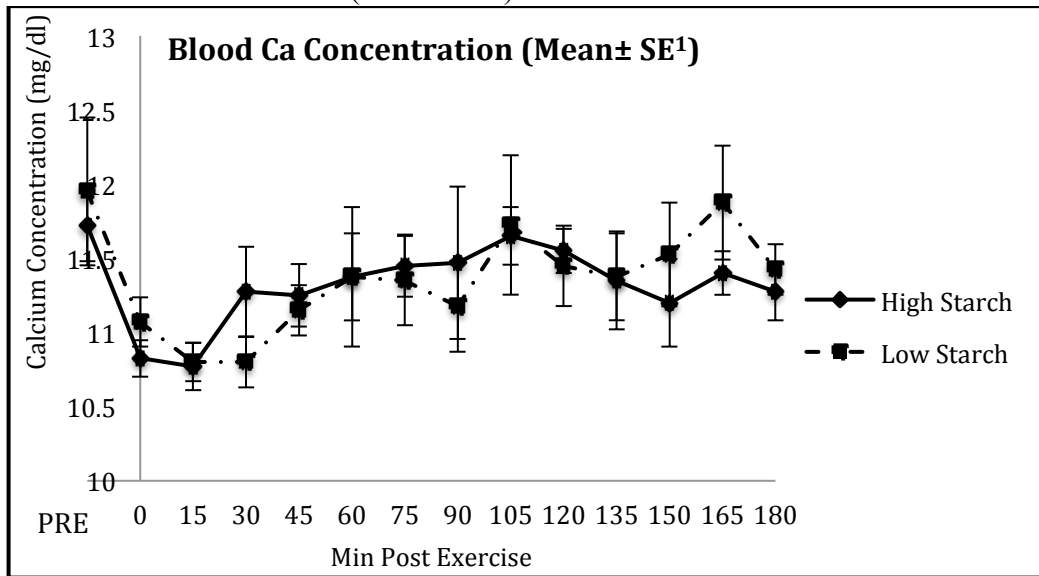
CHAPTER V

DISCUSSION AND CONCLUSION

Recovery from fatigue is a significant economic and health aspect of the performance horse industry. Equine athletes significantly deplete skeletal muscle glycogen stores by performing repeated bouts of highly intense exercise (Lacombe et al., 1999). Glycogen replenishment is a marker of recovery from intense exercise in both horses and humans (Waller and Lindinger, 2010). Compared to humans, however, horses are relatively slow at replenishing skeletal muscle glycogen stores. This slow rate of recovery can significantly affect equine performance, particularly at competitions where multiple bouts of highly anaerobic exercise are asked for within a 24 to 48 h period. Anecdotally, the issue of premature fatigue in performance horses has been compounded by the recent rise in popularity of low starch concentrate feeds. The anecdotal evidence of horses on low starch concentrates displaying symptoms of acute fatigue in the latter rounds of performance horse competition led to the primary objective of this project, which was to compare post exercise glycogen replenishment patterns and other markers of recovery in horses fed high and low starch diets.

Heart Rate

Although there was not a significant effect of treatment on HR, it is interesting to note the consistent trend where the horses on the LS diet had slightly higher heart rates throughout the exercise test. This trend has not been noted in similar investigations (Topliff et al., 1985 and Pagan et al., 1987). The cause of this trend could be related to an increase in glycolytic activity (noted in the trend in the increase of glycogen break down in

horses on the HS diet) or other metabolites released into the bloodstream. This increase in solute concentration could have increased the stroke volume, thereby making each heart beat more "efficient;" each contraction would have moved more blood to the tissues so the heart would have beat at a slower rate (Brooks et al., 299-308). To date, very few, if any, researchers have investigated the effect of diet on stroke volume in exercising horses.

Resting, Pre-Exercise Glycogen Concentration

There were no significant differences among treatments in the resting glycogen concentration, taken 12 h pre-SET. This is a different outcome than what was seen in similar experiments done by Topliff et al. (1983) and Pagan et al. (1987), who both saw significant differences in resting glycogen concentration between horses fed a high-starch control diet, and both a high protein diet and a high fat diet. This difference may be explained by the fact that the diets fed in this experiment only differed in starch concentration, rather than testing the affect of increasing another nutrient. Also, in both of these studies, the horses were being conditioned while on different diets so it is plausible that the statistical significance seen was a conditioning effect on top of a treatment effect. Finally, a possible explanation for the lack of significant difference among treatment was the small number of samples taken (4) and the large variations in concentration among samples. This sample-sample variation at the same time points may be due to inherent differences between horses. All of the sample concentrations fell within an acceptable range of concentrations compared to data collected by Topliff et al. (1983) and Lacombe et al. (2004).

Glycogen Depletion from SET

The SET used to deplete skeletal muscle glycogen in this project was designed to deplete both aerobic and anaerobic muscle fibers by having the horses trot for 30 min then working them at gradually increasing intensities on a high-speed treadmill. The increases in intensity were achieved by increasing speed and incline of the treadmill. After exercise, horses in both treatments had an approximate 50% decrease in resting muscle glycogen concentration. The combined works of Davie et al. (1996), Topliff et al. (1983) and Lacombe et al. (1999) show that a horse needs at least a 40% depletion of skeletal muscle glycogen to affect performance. Therefore, the SET used successfully depleted all four horses of glycogen to an acceptable point of fatigue.

This result contrasts with Waller et al. (2009), McCutcheon et al. (1999) and Pagan et al. (1987) because the SETs used in these investigations, similar to the one used in this project, only depleted 20-30% of the resting glycogen concentration. Lacombe et al. (1999), Davie et al. (1996), Geor et al. (2006), and Urschel et al. (2010) depleted 40-60% of the resting glycogen concentration of the gluteus medius by having horses perform repeated sprints, instead of gradually increasing intensity, on a high speed treadmill. Although the SET used here was perhaps less comparable to an equine competition than an SET involving repeated sprints, this SET was very successful at depleting skeletal muscle glycogen for the purpose of studying replenishment in the biceps femoris.

The SET used in this project was more successful than similar reported SET's because of the extended warm-up (designed for type I muscle fiber depletion) and the highly intense treadmill portion (that depleted type II fibers). The biceps femoris is also

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highly active in high limb extension, potentially causing it to utilize more glycogen to power contraction during both phases of exercise.

Glycogen Replenishment

There was a significant time x trt interaction in the skeletal muscle glycogen concentration taken after 48 h after exercise. The lack of statistical significance due to treatment at the other time points can be attributed to the low sample size and inherent horse-horse (sample-sample) variation, which yielded a lack of statistical power in the analysis. However, the greater concentration in the HS diet at 48 h post exercise shows a greater rate of replenishment on a high starch diet.

This result is supported by a significant increase in glycogen concentration at 6 hours on the HS diet, but no increase on the LS. Although there was no treatment x time effect at this time point, the time effect at 6 h shows a more rapid rate of early skeletal muscle glycogen replenishment when horses were fed the HS diet.

Compared to the results of Pagan et al. (1987) and Lacombe et al. (2004), the overall rate of glycogen replenishment in both treatments was relatively high, with the majority of horses replenishing skeletal muscle glycogen within 24 h of the exercise test. However, the relative values of glycogen concentration (determined as a ratio to the resting concentration) are in agreement with values obtained on horses supplemented with acetate post exercise found by Waller et al. (2009b).

The relatively rapid and consistent peak in glycogen concentration across both treatments, particularly the low starch treatment at 24 h post exercise may also be due to the fact that horses received a concentrate meal approximately 12 h before the 24 h-

sample was taken. This sample would have provided substrate for glycogen synthesis and elicited an acute insulin response that would have signaled an increase in glycogen synthesis prior to the sample time (Lacombe et al., 2003). The meal would have served as a post-exercise supply of metabolites for a super-compensation effect that has been seen in humans. To almost double resting glycogen concentration prior to a competitive event, human athletes will exercise to exhaustion, then supplement carbohydrate (Jentjens and Jeukendrup, 2003). The same phenomenon was observed here, to a lesser extent, on the LS diet.

Horses on the LS diet decreased in skeletal muscle glycogen concentration at the 48 h time point. None of the horses were exercised between the 24 and 48 hr time points, which makes the decrease in concentration more interesting, particularly because a similar trend has not been described in the literature. However, the decrease in glycogen concentration may not be an exercise-induced depletion, but an effect of the super-compensation effect seen at the 24 h sample. Essentially, due to a final rehydration and meal prior to the sample, the glycogen concentration increased at 24 hours. Although there was a meal roughly 12 h prior to the 24 h sample, there was no recovery and rehydration effect to drive glycogen synthesis.

The biceps femoris contains a greater distribution of muscle fiber types (Stull and Albert, 1981) compared to the commonly sampled gluteus medius. This may make it more metabolically active during exercise and subsequent recovery, which could cause a more rapid depletion and subsequent replenishment of skeletal muscle glycogen under both treatments. Lacombe et al. (2003) stated that Type I and IIa fibers (depleted by long, slow

exercise) are replenished before Type IIb fibers. It is plausible that some of the samples showed an increased relative replenishment (contributing to the horse-horse variation and a fast early rate of replenishment) because of a high concentration of I and IIa fibers in the samples and sub-samples.

Blood Glucose Concentration

One of the popular theories about glycogen metabolism is that glycogen replenishment is a correlated with substrate availability or blood glucose concentration. The blood glucose concentrations taken 0-180 min after exercise are within the range of acceptable concentrations in horses (Hoffman et al., 2003a). Although there was no significant effect of treatment on glucose concentration, there was a time effect on glucose concentration, lending credence to the theory that glycogen replenishment involves the clearance of the exercise-induced spike in glucose concentration. It appears there is a potential trend of an increased rate of glucose clearance in horses fed a high starch diet particularly between 30 and 90 min post exercise. This trend agrees with the data collected by Hoffman et al. (2003a) where horses on a high starch diet had an increased rate of response to insulin. As a result, the observed trend bears further investigation in a follow up study with greater statistical power.

Blood Lactate Concentration

Lactic acid production is indicative of anaerobic, and typically glycolytic metabolism at high intensity exercise. Although we did not collect samples for lactate analysis throughout exercise, the almost 20-fold ($P<0.05$) increase in lactate concentration pre-exercise to post-exercise indicates that horses where highly anaerobic and therefore

glycolytic at the end of exercise. There was no effect of treatment on lactate recovery and there was less sample variation at the same time points, which indicates that horses did not gain or lose fitness throughout the project (Rainger et al., 1994).

Total Plasma Protein and Ca Concentration

The concentrations of total plasma protein and Ca obtained from the post exercise recovery period indicate mild dehydration after exercise rather than a musculoskeletal or metabolic disorder (Carlson, 1995). There was no significant treatment or sample time differences in concentration for either total plasma protein or Ca.

Total protein was measured as a marker of hydration status throughout the 3 h post exercise recovery period. Under both diets, the total protein concentration was highest immediately after exercise, which shows that horses were dehydrated after exercise. By the 15 min post exercise time point the total protein concentration was back to the resting level, which indicates that horses physiologically increased their plasma volume (therefore, decreasing relative concentration of plasma protein) by moving water from the muscle tissue back to circulation. It is interesting to note a slightly, but not significantly higher average total protein concentration in horses on the LS diet, compared to the HS. This may indicate that horses on the LS had a smaller stroke volume, and could explain the consistent trend of higher HR throughout exercise on the LS compared to the HS.

Ca is an important second messenger ion in muscle contraction (Brooks, 187), so it would be interesting to examine the effect of dietary starch on Ca ion concentration during exercise. Here, several factors would likely affect the concentration including hydration status, metabolic status and fitness. In examining the data, while noting the lack of

significant interactions between both sample time and treatment it is interesting to note that the high starch treatment yielded a consistently higher Ca concentration than the low starch. This trend may be an effect of hydration status or feed and muscle or feed and gastrointestinal tract interaction.

Conclusion

Horses require greater than 553.7g starch/d for optimal recovery from intense exercise. Specifically a low starch diet results in an overall slower rate of skeletal muscle glycogen replenishment until at least 48 h post exercise. Further exploration is needed to determine other consequences of a low starch diet in the performance horse, but there may be an effect on heart rate during exercise and glucose clearance, immediately post exercise. The current results indicate that dietary starch is a significant component of recovery from and preparation for intense exercise.

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