EFFECT OF DIETARY MAGNESIUM STATUS ON INDICES OF MUSCULAR DYSFUNCTION IN EXERCISING HORSES

A Thesis

by

CASSIDY ANNALIESE KURTZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Dennis H. Sigler
Committee Members,	Clay A. Cavinder
	James Fluckey
Head of Department,	Gary Acuff

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ABSTRACT

Effect of Dietary Magnesium Status on Indices of Muscular Dysfunction in Exercising Horses.

(December 2009)

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Throughout the performance horse industry, the occurance of various muscle disorders is common and can be detrimental to the performance and longevity of equine athletes. Research has revealed effects of diet manipulation, exercise, and electrolyte supplementation on the symptoms and occurrence of disorders like exertional rhabdomyolysis (ER). However, there has been no investigation on effects of Mg on muscle function in horses during exercise.

Six Quarter Horse mares were used to study the effects of varying levels of Mg on indices of muscular dysfunction during a standardized exercise test (SET) on a high-speed treadmill. Three rations were used over three 28 d periods: control (Trt 1), low Mg (Trt 2), and high Mg (Trt 3). A baseline SET was conducted prior to day 0 (Trt 0). Blood samples were taken during the SET at rest, immediately post, 1 h, 6 h, and 24 h post exercise for analyses of serum muscle enzymes and Mg concentrations. Heart rates (HR), respiration rates (RR), and rectal temperatures (RT) also were documented.

No effect of Trt was observed on HR or RR at any point throughout the SET. Resting RT's were lowest in Trt 0 ($P<0.05$). There was no Trt effect on blood lactate (LA) during the SET; however, blood glucose (GLU) at rest in Trt 2 was lower than Trt 0 ($P<0.05$). Treatment had an effect on both resting serum creatine phosphokinase (CK), as Trt 3 was higher than Trt 0, and the change in CK from rest to 24 h post SET, where Trt 1 exhibited the greatest increase in CK concentration ($P<0.05$). Serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were not affected by Trt ($P>0.05$). Serum alkaline phosphatase (AP) at rest and 6 h post exercise was lower in Trt 2 than in Trt 3 ($P<0.05$). Additionally, serum P was lowest at rest in Trt 0 and highest 6 h post exercise in Trt 3, also varying within each Trt. Average daily intake (ADI) of Mg was higher in Trt 1 and 3 than in Trt 2 ($P<0.05$). Finally, at rest, immediately post and 24 h post exercise, serum Mg was highest in Trt 3 ($P<0.05$).

Results suggest there is an effect of dietary Mg on serum muscle enzyme and Mg concentrations and potentially, overall performance in the equine athlete.

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NOMENCLATURE

ALB	Albumin
BUN	Blood Urea Nitrogen
CRE	Creatine
DB	Direct Bilirubin
GGT	Gamma Glutamyltransferase
GLOB	Globulins
GLU	Glucose
HR	Heart Rate
LA	Lactate
PSSM	Polysaccharide Storage Myopathy
RR	Respiration Rate
RER	Recurrent Exertional Rhabdomyolysis
SET	Standardized Exercise Test
RT	Rectal Temperature
TB	Total Bilirubin
TSP	Total Serum Protein
Trt 0	Treatment 0 – Baseline SET prior to day 0
Trt 1	Treatment 1 – Control Diet
Trt 2	Treatment 2 – Low Mg Diet
Trt 3	Treatment 2 – Mg Supplemented Diet

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CHAPTER I

INTRODUCTION

As the equine industry evolves, higher purses and greater competition continue to place increased exercise intensity and demand on equine athletes. With such intense exercise, horses can exhibit a variety of symptoms relating to muscle soreness, cramping, breakdown, and even an increase in recovery time. As a result, competitive horses under high demands are unable to perform at their potential thus influencing their overall profitability and longevity. While numerous studies have documented effects of training methods, diet manipulation, and supplementation on equine muscular function during exercise, further research is warranted to examine other possibilities.

During severe muscle cramping or breakdown, a form of exercise-induced myopathy can be responsible. Across all disciplines, exertional rhabdomyolysis or "tying up," is a common muscle disorder which can negatively affect a horse's athletic performance. Substantial evidence supports modifying diets to include low starch and high fat, maintaining proper electrolyte levels, and regular exercise as solutions that may decrease episodes of tying up (Valberg et al., 1998, 1999b; De La Corte et al., 1999a, b, c; MacLeay et al., 1999a; Valentine et al., 2001b; McKenzie et al., 2003a; Ribeiro et al., 2004). However, in equine studies there has been little work done with regard to the potential effects of dietary magnesium on muscle function during exercise.

This thesis follows the style of the Journal of Animal Science.

Magnesium is an activator of over 325 metabolic enzymes and reactions that involve energy production, ion transport, conduction of nerve impulses and muscle contraction (Newhouse and Finstad, 2000). Widespread investigation proposes Mg acts specifically in the regulation of intracellular Ca channels and thus has the ability to cause prolonged contraction without relaxation (Romani, 2006; Fontenot et al, 1989). However, in humans extensive research reveals confounding results regarding the effect of Mg on muscle function and exercise performance. It has been suggested that individuals in a state of Mg deficiency prior to supplementation, are capable of increased performance, whereas athletes without prior Mg deficiencies experience no effect of supplementation (Liu et al., 1983; Nielsen and Lukaski, 2006). Regardless, the role of Mg in the muscle contraction process, as well as its probable influence on athletic performance, warrants further research in the equine model.

Therefore, after review of available literature, the focus of this study is to examine the effects of supplemental dietary Mg on elevations of serum muscle enzymes following the onset of exercise. The hypothesis is that supplemental dietary Mg will positively influence serum muscle enzymes by decreasing cellular damage and further, improve muscle function during exercise. Such benefits could improve the profitability and longevity of the career of the equine athlete by allowing them to perform more effectively and decreasing the likelihood of injury. Thus, the primary objective of this study is to evaluate the effect of varying levels of dietary Mg on muscle contractile properties in exercising horses.

CHAPTER II

REVIEW OF LITERATURE

Equine Muscle Disorders

Various muscle disorders characterized by the presence of pain and stiffness following exercise are common across multiple breeds of horses today. While numerous designations have been assigned to such disorders, Equine exertional rhabdomyolysis (ER), often referred to as "tying up" or Monday morning disease, specifically describes a condition affecting both the skeletal muscles and muscles of the heart shortly after the onset of exercise. In the event of repeated episodes occurring in performance horses, ER may cause a significant limitation in athletic potential. Within the past 20 years, advances in research have uncovered several possible causes of this syndrome varying from deficiencies in vitamins, minerals and electrolytes to genetic predisposition, temperament and inconsistent exercise regimens. Regardless of cause, extensive documentation confirms ER can be categorized as sporadic or chronic with chronic cases subdivided into 2 heritable forms, recurrent exertional myopathy (RER) and polysaccharide storage myopathy (PSSM) (Valberg et al., 1999b).

Polysaccharide Storage Myopathy. Polysaccharide storage myopathy is essentially a glycogen storage disorder that is suggested to be prevalent in 6 to 12% of healthy Quarter Horses in the United States (McCue and Valberg, 2007). Typically, this syndrome is characterized by increased muscle glycogen concentrations and an accumulation of amylase-resistant polysaccharide inclusions in skeletal muscle fibers

(Valberg et al., 1999b). Muscle glycogen concentrations in PSSM horses have been noted to be 2 to 3 times higher than those found in unaffected horses (Valberg et al., 1999b), however accumulation of muscle inclusions may be a gradual process and one not noted until horses reach 2 years of age (Valberg et al., 1997; De La Corte et al., 2002).

Previous research has partially uncovered the mechanisms responsible for enhanced glycogen storage in horses with PSSM. As is evident in humans, glycogeneses result from an inability of muscle to use glycogen, therefore greatly impairing oxidative metabolism (Sahlin et al., 1995). However, no such defects in glycolytic or glycogenolytic pathways have been noted in PSSM horses (Valberg et al., 1998; Valentine et al., 1998). Polysaccharide storage myopathy identified horses have demonstrated higher glycogen utilization rates compared to unaffected horses during anaerobic exercise. Therefore, there is speculation that an accumulation of glycogen in the skeletal muscle is not a utilization problem, but rather a regulatory defect that affects glycogen synthesis. Horses with PSSM exhibited a higher rate of glucose clearance following administration of an IV bolus compared to healthy control horses, resulting perhaps from increased insulin sensitivity (De La Corte et al., 1999b). Additionally, not only did PSSM affected horses demonstrate lower insulin concentrations at rest and after IV administration of glucose over control horses, they too exhibited a significant decrease in blood sugar immediately following an insulin IV dosing, compared to control horses (De La Corte et al., 1999a). Two additional studies were reported. After consumption of a sweet feed meal, both blood glucose and insulin concentrations were

lower in affected horses than healthy horses (De La Corte et al., 1999c). Annandale et al. (2004) also revealed a two-fold increase in glucose clearance in affected horses over healthy horses during an insulin sensitivity test via a euglycemic hyperinsulinemic clamp. Thus, it is apparent PSSM horses exhibit enhanced glucose clearance and insulin sensitivity over non-affected horses. Skeletal muscle in light breed horses comprises almost 50% of BW, making it the largest mass of insulin-responsive tissue in the body (Geor, 2005). Although no published data on glucose uptake in skeletal muscle exists, it is reasonable to speculate that PSSM affected horses also may have an increased capacity for glucose uptake as a result of both the disorder and the presence of such a large tissue mass.

Numerous studies indicate the expression of PSSM can be modified via a change in dietary energy source. Horses with PSSM receiving minimal exercise and moderate to large amounts of concentrates with a high nonstructural carbohydrate content appeared to have more frequent and severe signs of ER (Valberg et al., 1997; Valentine et al., 2001b). Consequently, a decreased frequency and severity of ER episodes was observed with the feeding of a diet low in hydrolyzable carbohydrate and high in fat (Valentine et al., 2001b; McKenzie et al., 2003b; Ribeiro et al., 2004). While there is controversy over whether the improvement of PSSM horses is a result of a dietary decrease in nonstructural carbohydrates or an increase in fat, it is important to provide an altered diet that meets DE requirements to help manage affected horses. Just the same, regular exercise also may have the ability to prevent excessive muscle glycogen storage in horses with PSSM, as 75% of ER episodes in affected horses were eliminated with the

implementation of a low soluble carbohydrate, fat added diet paired with daily exercise (Valberg et al., 1998, 1999b).

Recurrent Exertional Rhabdomyolysis. Recurrent exertional rhabdomyolysis (RER) is a heritable disorder most commonly affecting Thoroughbreds whereby high levels of stress and excitement result in excessive muscle contraction and even necrosis due to a defect in intracellular Ca regulation (Lentz et al., 1999; MacLeay, 1999b; Valberg, 1999b; McKenzie et al., 2003b). *In vitro* testing on skeletal muscle from RER affected Thoroughbreds demonstrated a hypersensitivity of the muscle to agents responsible for inducing the Ca^{+} release from the sarcoplasmic recticulum. For example, lower concentrations of such agents (ie:caffeine), are required to induce muscle contraction in horses with RER as opposed to those without (Lentz et al., 1999). However, confounding results between studies in humans and RER horses leave the likely causative gene mutation responsible for increased sensitivity and Ca release unidentified (Hinchcliff et al., 2004). Regardless, the highest prevalence and severity of RER episodes have been noted in 2-to-3 year old fillies with nervous temperaments, although sex predilection fails to be obvious in older RER horses (MacLeay et al., 1999a).

As with PSSM, manipulation of dietary energy source has been well documented as a successful method for modulating temperament and expression of muscle damage in Thoroughbred horses with RER (MacLeay et al., 2000; McKenzie et al., 2003a). Again however, the effect of energy source greatly depends on daily dietary energy (DE) intake (Geor, 2005). In a study involving RER affected Thoroughbreds, a diet providing 21.4

Mcal/d with the majority of energy being supplied via fat or hydrolyzable carbohydrate, showed no difference in post-exercise muscle necrosis as indicated by serum CK activity. However, when the affected horses were provided a diet high in sweet feed totaling 28.8 Mcal/d, a DE intake similar to that of racehorses, significant increases in post exercise serum CK activity was observed (MacLeay et al., 2000). Further, in a crossover study by McKenzie et al. (2003a), RER horses were fed both a diet high in hydrolyzable carbohydrate content and sweet feed as well as a diet low in hydrolyzable carbohydrate content with added fat. Both diets provided 28.8 Mcal/d and serum CK activity was increased when horses were fed the diet high in hydrolyzable carbohydrate, but not when fed the low diet with added fat.

Although the mechanism responsible for exercise-induced muscle damage in RER horses fed high hydrolyzable carbohydrate diets is unclear, it has been suggested that there may be a relationship between dietary energy source and behavior. Historically, horses fed a high fat diet with low nonstructural carbohydrates have demonstrated a calmer demeanor and modulated nervousness, both possibly being the contributing factors to a decrease presence of ER episodes (MacLeay et al., 1999a; McKenzie et al., 2003a). Further, in rats that were provided a high sucrose diet, enhanced corticotrophin releasing hormone expression in the hypothalamus paired with stimulation of the sympathetic nervous system was observed, thus resulting in increased levels of stress and anxiety (Levine et al., 2003).

Extensive documentation of ER reveals a disorder, whereby a defect in muscle metabolism causes cramping, or in severe cases, contraction of the muscle without the

ability to relax. Typically, immediately following the start of exercise, horses with ER will exhibit pain and firmness in the loin, croup, and large gluteal muscles. Excessive sweating, rapid heart rate, increased respiration, and muscle tremors may also be noted. In extreme cases, horses may be reluctant to move and may produce red-colored urine due to the release of myoglobin from damaged muscle cells (McKenzie and Firshman, 2009).

Diagnosis of ER can be confirmed with elevated levels of blood serum lactate dehydrogenase (LDH), creatine phosphokinase (CK), aspartate transaminase (AST), all indicative of muscle cell damage (Hinchcliff et al., 2004). Additionally, biopsies taken from the mid-gluteal and semimembranous muscles, largely composed of type-II (slow twitch) fibers permit specific diagnosis of various forms of chronic ER. Regardless of form, ER affected muscle cells will exhibit vacuolization and fragmentation of myocytes as well as centrally located nuclei signifying possible myocyte regeneration, fibrosis and macrophage infiltration (Ulman and Lacey, 2000).

Diagnosing Muscle Disorders Using Serum Chemistry

Blood analysis continues to be a generally accepted and commonly utilized tool for evaluating health and disease in humans and animals alike. However, while the blood of an equine athlete reveals an abundance of information, it can be significantly altered by a vast number of factors. Therefore, great care must be taken in analyzing and interpreting blood work when assessing health status of horses.

Aspartate Transaminase. Most notably found in the liver, skeletal and cardiac muscle of the horse, aspartate transaminase (AST) is an enzyme with a long half-life whereby noted values may represent changes over several days or weeks. Peak values of AST are typically observed 24 to 36 hours post exercise and are representative of muscle strain or damage. However, horses in training frequently exhibit a plateau of AST levels as they become more conditioned (Marcella, 2009). Further, horses suffering from ER can show normal AST levels regardless of extensive muscle damage, as the levels can be slow to peak (Valberg et al., 1999b). Significantly elevated AST levels (50,000 IU) above normal values of 300 to 360 IU are sometimes observed in both clinically normal horses and ER affected horses, leaving a direct correlation between ER and AST levels unclear. Consequently, low AST levels may be indicative of too little work from the horse compared to its present state of fitness. Either way, AST should be analyzed along with gamma glutamyltransferase (GGT) in order to confirm any muscle damage (Marcella, 2009).

Lactate Dehydrogenase. Otherwise known as LDH, lactate dehydrogenase is frequently released by both cardiac and skeletal muscle during times of stress. Along with AST, LDH can be useful in pinpointing muscle dysfunction. Since individual isoenzymes of LDH exist, pairing these levels with other muscle enzymes is required in order to accurately place diagnosis. There is suggestion that LDH may indicate nothing other than intense or prolonged exercise (Marcella, 2009).

Creatine Phosphokinase. Found in skeletal, smooth, and cardiac muscles in high concentrations, CK is important in energy production as it catalyzes the conversion of creatine phosphate and ADP to creatine and ATP (Bagshaw, 1993). Depending on the type and duration of exercise, release of CK is thought to be due to an alteration in membrane permeability or skeletal muscle damage (Volfinger et al., 1994). Generally, in plasma or serum, CK activity will peak 4 to 6 hours post exercise (MacLeay et al., 2000). Increases in CK resulting from exercise have been recognized in humans and sled dogs (MacLeay et al., 2000; Valentine et al., 1998). Additionally, persistent or intermittent increases of CK, potentially 10 times above the normal range of 80 to 120 IU, are again said to be suggestive of cellular death or myopathy (Burr et al., 1997; Windebank and Mulder, 1996). Furthermore, Marcella (2009) proposed high levels of AST and CK in a horse that has not undergone intense exercise directly implicates muscles as the primary site of dysfunction. Thus, analysis of CK levels in exercising horses proves to be advantageous in identifying ER episodes.

Blood Urea Nitrogen and Creatinine. Used principally to evaluate kidney function, blood urea nitrogen (BUN) is commonly paired with creatinine to observe the clearance of myoglobin from a horse's system. Increased BUN concentration can be influenced by pre-renal and post-renal factors, while increased creatinine is typically representative of kidney cell damage. As documented, in severe cases of ER myoglobinuria and renal failure is evident (McKenzie and Firshman, 2009).

Electrolytes. Evaluation of electrolytes is imperative to evaluating exercise tolerance and other work related stressors. Sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), and calcium (Ca) imbalances can crucially affect muscle function, cellular metabolism, enzyme action, and nerve conduction (Marcella, 2009). Additionally, decreased performance and metabolic illness may result in the case of severe imbalances, as published reports noted improvement in horses following a correction of electrolyte clearance ratios (Hinchcliff et al., 2004). Low K concentrations are of specific interest since episodes of rhabdomyolysis in humans have occurred in the presence of low K levels (Shintani et al., 1991). However, conflicting results in ER horses with K deficiencies make it hard to draw a direct correlation between the two (Beech et al., 1993; Shintani et al., 1991). In comparison to controls, Thoroughbreds administered furosemide and sodium bicarbonate exhibited lower plasma Cl, Mg, K, and Ca concentrations. Further, following exercise CK levels appeared significantly higher in those horses treated compared to the controls (Freestone et al., 1991).

Magnesium Research in Humans, Horses, and Other Animals

Chemistry. Magnesium (Mg) is a divalent metal ion and represents the fourth most common cation in the body (Frausto da Silva et al., 1991). Specifically, as the second most abundant intracellular ion, 30 percent of Mg^{+2} is found in muscle tissue (Grace et al., 1999). The primary role of Mg in the body is to complex highly charged anions for the facilitation of enzyme-substrate interactions or stabilization of the polymer conformation (Frausto da Silva et al., 1991). Therefore, Mg is an essential mineral as it

is a co-factor for over 325 enzymatic reactions involving cellular energy production and storage, protein synthesis, adenylate cyclase synthesis, maintainance of cellular electrolyte composition, and stabilization of mitochondrial membranes (Newhouse and Finstad, 2000).

Storage and Transport. Within the cell, Mg can be found in the nucleus, mitochondria, endoplasmic reticulum, and cytosol (Cowan, 1995). The majority of this fraction is bound to proteins, adenosine triphosphate (ATP) and adenosine diphosphate (ADP). Further, approximately 1 percent of the intracellular Mg is considered free ionized Mg and remains such due to the limited permeability of the cell membrane to Mg and transport systems which regulate the rate of Mg in and out of the cell (Romani et al., 1993). The transport of Mg out of cells may be coupled to Na transport, which is ATP dependent, whereas the transport of Mg into the cell may be linked to Na or bicarbonate transport (Rude, 2000). Regardless, hormonal and pharmalogical influences may impact the transport of Mg, although specific mechanisms have not been completely explained (Gunther, 1993).

Function of Magnesium. The primary cellular role of Mg involves anion charge neutralization where specifically, Mg functions to stabilize ATP in ATP-dependent enzymatic reactions (Shils, 1994). As a result, Mg is vital for efficient metabolism of carbohydrates, lipids and proteins, as well as the synthesis of mitochondrial ATP (Rude, 2000).

Cell activity is regulated by guanine nucleotide-binding (G) protein-mediated cascade reactions and further, the adenylate-cyclase (AC) system via hormones,

neurotransmitters and cellular effectors (Rude, 2000). Vital to this system, Mg is specifically required in the actual activation or inhibition of AC, as the necessary substrate is Mg-ATP (Rude, 2000). Furthermore, AC affects the Ca concentration in the cytosol of the cell, as it is a component of a second messenger system that functions to regulate Ca release. Activation or down regulation of AC via Mg-ATP, causes intracellular Ca concentrations to fluctuate, thereby influencing skeletal and smooth muscle contraction.

Magnesium also regulates other cellular proteins, including ion channels, through the cell membrane which allow passage of ions across the cell surface. In particular, Mg can bind internally or externally to the mouth of ion channels, thus obstructing passage or modifying channel gating and further, resulting in its opening or closing (Romani, 2006). Similarly, ATPase dependent ion pumps transporting ions across cell membranes can be inhibited via depletion of Mg, thus impairing their pumping abilities. Therefore, along with channel modulation, Mg plays a role in channel availability and total number of functional channels (Fakler et al., 1994).

Absorption. Magnesium absorption from commonly used feedstuffs containing 0.1 to 0.3 percent Mg, appeared to be 40 to 60 percent (Hintz and Schryver, 1972; Meyer, 1979). According to Kapusniak et al. (1988), Mg abosorption occurs primarily in the small intestine. Common inorganic supplemental sources include magnesium oxalate, magnesium sulfate, and magnesium carbonate (Harrington and Walsh, 1980). Although studies in humans indicate a decrease in absorption of inorganic sources of Mg, specifically magnesium oxide, (Lindberg et al., 1990; Walker et al., 2003),

Harrington and Walsh (1980) observed supplementation of inorganic Mg sources (magnesium oxide) increased Mg absorption in growing foals (>70 percent). Additionally, McKenzie et al. (1981) reported the digestibility of Mg to be 42 to 45 percent and further, not affected by oxalate. Inclusion of other minerals also has been documented to affect Mg absorption. Excess phosphorous decreased Mg absorption (Kapusniak et al., 1988), and high K concentrations were reported to decrease Mg digestibility as well (Weidenhaupt, 1977). Further, in humans, Mg absorption increased as dietary Mg decreased, or Mg status was poor, thus illustrating an inverse proportion of intake to absorption (Fine et al., 1991).

Excretion. Magnesium balance is controlled by the kidney; therefore any Mg not retained by the body will consequently be removed via the kidney (Bohl and Volpe, 2002). During periods of low Mg intake, the kidneys effectively reabsorb and conserve Mg (Wester, 1987), whereas supplementation increases urinary excretion while maintaining normal serum levels (Groff and Gropper, 2000). This is further supported by Meyer and Ahlswede (1977) who documented Mg intake of 5 to 6 mg/kg of BW/d decreased renal excretion of Mg, whereas intake of 20 mg/kg of BW/d induced normal serum Mg values.

Magnesium Requirements. At the assumed absorption rate of 40 percent, a 500 kg horse at maintenance requires 15 mg/kg of BW/d (NRC, 2007). During early lactation however, as well as under moderate to intense exercise, a horse's Mg requirement increases 1.5 to 2 times beyond maintenance (Stewart, 2004). Thus, the Mg requirement for light work is documented at 19 mg/kg BW, 23 mg/kg BW for moderate

work, and 30 mg/kg BW for heavy work, respectively (NRC, 2007). While Mg requirements suggested for horses in race training are contradicting (Pagan, 1994; Stephens et al., 2004), the requirements of Mg are reported to be influenced both by exercise and stage of training (Stephens et al., 2001). Therefore, careful analysis and proper balance of the diet of active horses diet to meet necessary nutritional requirements remains imperative to maintain proper body function.

Deficiency and Acid-Base Balance. Symptoms resulting from a dietary Mg deficiency have been reported in a number of animals. Harrington (1974) reported hypomagnesaemia in foals with Mg intake at 7-8 mg/kg of diet/d. Similar results were observed by Meyer and Ahlswede (1977) when Mg intake was equivalent to 5-6 mg/kg of BW/d. In ruminants, low concentrations of Mg in forage were reported to possibly induce grass tetany, a condition somewhat similar to hypomagnesaemia in monogastrics (Grunes and Welch, 1989). Furthermore, forage low in both Mg and Ca concentrations was even more likely to induce grass tetany (Grunes et al., 1970). Clinical signs of a Mg deficiency include nervousness, muscle tremors, ataxia with the potential to cause death (NRC, 2007). Outside of a low dietary intake of Mg, electrolyte imbalance may also play a role in cases of hypomagnesaemia and excercised induced myopathies in horses as well as other species (Freestone et al., 1991).

A severe distortion of the acid-base balance system influenced by an excessive intake of K has been implicated as a causative factor of death in cattle and hypomagnesaemia in sheep (Neathery et al., 1979). Further, muscular tremors of legs and excitability were observed in calves receiving doses of K greater than 0.58 g/kg BW

(Neathery et al., 1979). In heifers, tetany akin to that observed in cases of field grass tetany was induced via administration of KCl paired with either citric or trans-aconitic acid (Bohman et al., 1969). While blood Mg levels were not analyzed in the above studies, Kunkel et al. (1953) documented significant decreases in serum Mg upon the inclusion of potassium bicarbonate in diets of sheep. However, although hypomagnesaemia was observed, no tetany occurred; further suggesting severity of the hypomagnesaemia was not great enough to produce typical convulsions. Most importantly, the reduction in serum Mg after an increase in dietary K warrants that low blood Mg could be the product of imbalances in the acid-base system. Further, actual deficiencies in Mg may alternatively occur from inadequate absorption of the mineral from the digestive tract (McDonald et al., 2002).

Interaction Between Magnesium and Exercise. The effect of exercise on Mg requirements, utilization, as well as effects of a Mg deficiency on performance has been clearly documented (Nielsen and Lukaski, 2006). Exercise is a potent stressor and appears to lead to Mg depletion via increased excretion through sweat and urine, as well as alterations in blood Mg levels (Bohl and Volpe, 2002). As noted, loss of intracellular Mg can lead to muscle weakness, neuromuscular dysfunction, cramping and spasms. Therefore, as studies have indicated in both animals and humans, a marginal Mg deficiency can compromise exercise capacity (Laires et al., 1989; Lukaski et al., 1983).

Magnesium's interaction with Ca in excitation contraction coupling processes illustrates its significant influence on proper muscle function (Larvor, 1983). As such, Mg may compete with Ca for binding sites on troponin C and myosin (Iseri and French,

1984). In the event Mg becomes bound to Ca binding sites, Ca is unable to bind and contraction is inhibited (Landon and Young, 1993). However, if muscle contraction is stimulated via Ca binding to troponin C and myosin, Ca must be pumped back into the sarcoplasmic reticulum to permit relaxation (Vander et al., 1980). Further, it is noted that in the event of dysfunction of the Mg-dependent Ca pump, the uptake of Ca is inhibited, thus resulting in sustained fibril contraction (Fontenot et al., 1989).

Effect of Magnesium Supplementation on Exercise. Published research suggests a Mg deficiency may reduce physical performance in humans, and further, Mg status may significantly influence exercise capacity (Bohl and Volpe, 2002). Magnesium supplementation to athletes in a variety of disciplines resulted in a 50 percent increase in endurance, faster performance times, delayed exhaustion, and increased strength (Bohl and Volpe, 2002). On the other hand, a number of similar studies found that Mg supplementation had no effect on exercise capacity.

Although there are conflicting results regarding Mg supplementation and the enhancement of performance, there is question as to whether or not increased Mg intake of physically active individuals is beneficial only in cases of individuals who are in a state of Mg deficiency prior to supplementation (Nielsen and Lukaski, 2006). Such has been observed in a case study of a female tennis player deficient in Mg as a result of increased loss through sweat. Results illustrated Mg supplementation relieved muscle spasms as well as raised serum Mg level of the athlete (Liu et al., 1983). While elimination of muscle spasms was not directly correlated to improved performance, such relief would allow an athlete to be active for a longer period of time. Interestingly

enough, supplementation of Mg to reduce muscle spasms and a hypomagnesaemia state also has been utilized in cattle which suffer from grass tetany. In these instances, high K levels in forage (>3% K), result in a Mg deficiency in the animal. Severe episodes of grass tetany are commonly treated via intravenous administration of Ca-Mg gluconate or a like solution (Bohman et al., 1969). Such treatment relieves muscle tremors or tetany while subsequently raising plasma Mg levels, therefore further supporting the idea that Mg influences muscle function.

CHAPTER III

EXPERIMENTAL PROCEDURE

Management of Animals

Quarter Horse mares (n=6), 3 to 9 yr old, owned by the Department of Animal Science at Texas A&M University were used in this study. Prior to the experiment, all horses were examined by a veterinarian to insure proper health and soundness. The mares were dewormed and vaccinated prior to use and throughout the study, according to a regular deworming and vaccination schedule. Horses were housed in individual stalls at the Texas A&M University Equestrian Center and managed in accordance with the approved guidelines laid out by the Institutional Agricultural Animal Care and Use Committee.

All mares were relatively untrained and unfit prior to the beginning of the trial. A 60 d training period was conducted to equilibrate the level of training and fitness across all subjects. During this period, mares also were taught to drive with driving lines and to pull a sled by way of a draft-type harness. Horses underwent training 3 d/wk for 30 min at a walk.

Experimental Design

This experiment was designed and conducted as a repeated measures study. The duration of the trial was 105 days consisting of three 28 d periods, followed by a standardized exercise test (SET) and a subsequent 5 d transition period whereby horses

were gradually switched onto a new concentrate ration. Each 28 d period served as a separate treatment, thus resulting in 3 treatments for the trial. Throughout all 3 treatments, the mares were fed the same batch of coastal Bermudagrass (CB) hay. The concentrate, specifically levels of Mg and K, was the only variable that changed from treatment to treatment. The treatments consisted of a control diet (Trt 1), a low Mg diet (Trt 2), and a Mg supplemented diet (Trt 3), all of which are discussed below. Body weights were recorded weekly and daily feeding amounts were adjusted to try to maintain constant body weight throughout the trial. All mares were kept on the same treatment throughout each period, to account for changes in physical condition during the experiment.

Experimental Diets

Three diets were utilized in the present study and served as Treatments 1, 2, and 3. The same CB hay was fed throughout the trial and analyzed to include 12.21% CP, with 0.13% Mg and 1.71% K respectively (Table 1). The concentrate portion of the diet was a commercially available grain based 12% crude protein textured sweet feed (Producer's Cooperation Association, Bryan, TX). The control diet (Trt1) contained 0.22% Mg and 1.06% K and the low Mg diet (Trt 2) contained 0.23% Mg and 1.81% K. Finally, the supplemented Mg diet for Treatment 3 contained 0.33% Mg and 0.81% K respectively (Table 2). All concentrate and hay samples were taken throughout the study, compiled and immediately sent off for analysis (SDK Laboratories, Hutchinson, KS).

Table 1. Analysis of coastal Bermudagrass hay (DM basis)

Component	Coastal Bermudagrass Hay
Moisture, %	8.08
Dry Matter, %	91.92
Crude Protein, %	12.21
Crude Fiber, %	30.83
Acid Detergent Fiber-ADF, %	36.62
Neutral Detergent Fiber-NDF, %	65.94
Fat-EE, %	2.42
Ash, %	6.53
Calcium, %	0.38
Phosphorous, %	0.19
Potassium, %	1.71
Magnesium, %	0.13
Sodium, %	0.03
Sulfur, %	0.16
Cobalt, ppm	<0.20
Copper, ppm	10.90
Iron, ppm	299.00
Manganese, ppm	78.80
Molybdenum, ppm	<0.30
Zinc, ppm	25.80

Table 2. Analysis of concentrate diets (DM basis)

	Treatment[1]		
Component	**1**	**2**	**3**
Moisture, %	13.76	12.86	11.90
Dry Matter, %	86.24	87.14	88.10
Crude Protein, %	16.00	15.14	13.49
Crude Fiber, %	5.68	6.41	6.61
Acid Detergent Fiber-ADF, %	7.87	7.63	8.56
Neutral Detergent Fiber-NDF, %	17.66	16.38	16.26
Fat-EE, %	6.36	7.44	5.65
Ash, %	5.71	7.83	6.20
Calcium, %	0.76	1.50	1.52
Phosphorous, %	0.55	0.58	0.42
Potassium, %	1.06	1.81	0.81
Magnesium, %	0.22	0.23	0.33
Sodium, %	0.22	0.29	0.22
Sulfur, %	0.31	0.25	0.23
Cobalt, ppm	0.72	1.40	0.87
Copper, ppm	40.10	39.00	29.60
Iron, ppm	112.00	153.00	311.00
Manganese, ppm	77.10	106.00	63.40
Molybdenum, ppm	<0.30	0.55	0.83
Zinc, ppm	71.90	127.00	75.20

[1]Treatments: 1=control; 2=low Mg; 3=high Mg

All mares were fed on average 1.7% BW per day, 1% of BW in hay and 0.7% of BW in concentrate. Therefore, each feeding consisted of 0.5% BW hay and 0.35% BW concentrate, respectively. Horses were fed individually in their stalls at 0600 h and 1700 h. Both concentrate and hay were provided at the same time and all subjects had *ad libitum* access to water. Any refusals present at the time of the next feeding were collected, weighed and recorded.

At the completion of each 28 d period and the subsequent SET, mares were gradually switched onto the new concentrate ration over a period of 5 d. The ration transition consisted of 2 feedings in which the concentrate portion of the ration included 25% new concentrate (ie: Trt 2) and 75% previous concentrate (ie: Trt 1). The next 4 feedings, or 2 d, were presented as 50% new concentrate and 50% previous concentrate. The fourth day of transition the ration included 75% new concentrate and 25% previous concentrate and by the fifth, and final day, all subjects were fed 100% of the new concentrate.

Exercise

The horses were exercised utilizing a full draft-type driving harness and wooden weighted sled every other day for a total of 3 d/wk. On off days, horses were turned out for 1.5 h of free exercise for a total of 3 d/wk. Three horses were worked Monday, Wednesday, Friday and the remaining 3 were exercised Tuesday, Thursday, and Saturday. On Sundays, all mares were given the day off for rest. Horses were taught to pull the sled and fitted with harnesses prior to the first day of exercise.

Exercise regimens throughout all treatments with the weighted sled included a 5 min warm-up with an empty sled, a 10 min workout with weight added to the sled via sand-filled plastic 18.9 L buckets of varying weights, followed by a 2 min cool down without sled or weight. The sled alone weighed 55.9 kg and buckets weighed either 11.4 kg or 22.7 kg. For the first 28-d period (Trt 1), the weighted exercise protocol included a 5 min warm up, 10 min workout with enough weight added to the sled to maintain a

heart rate (HR) of 120 to 130 bpm, and a 2 min cool down. At one point during the 10 min workout, horses were asked to reach a HR 150 bpm. Treatments 2 and 3 consisted of a 5 min warm-up, 10 min work-out with enough weight added to the sled to maintain a HR of 120 to 140 bpm, and a 2 min cool down. Again, at one point during the 10 min work-out, horses were asked to reach a HR of at least 150 bpm, or above one time. Heart rates were recorded at set times throughout every weighted exercise bout over the trial.

On off days, horses were turned out in individual 45 m diameter round pens for 90 min of free exercise. Turnout occurred simultaneously with the weighted work-outs in order to keep the time of exercise similar from day to day.

At the end of each 28-d period, horses were transferred to the Texas A&M University Horse Center for a 24 h period before being put through a 30 min SET on a high-speed treadmill (Säto, Uppsala, Sweden). The 24 h period was to allow for HR to return back to baseline levels before testing. The SET protocol included: 5 min at 1.8 m/s and 0% incline, 5 min at 2.9 m/s and 0% incline, 5 min at 2.9 m/s and 2.1% incline, 5 min at 5.2 m/s and 2.1% incline, 5 min at 5.6 m/s and 2.8% incline, and 5 min at 6.5 m/s and 2.8% incline. Horses traveled a total of 7,870 m over the duration of the test. Immediately following the 30 min SET, horses were removed from the treadmill and cooled down at a walk for 10 min before being returned their respective stalls. Each SET began at 0800 h and horses followed the same order in an attempt to keep environmental conditions comparable across all treatments. At the completion of the

SET, the mares were transported back to their primary housing at the Texas A&M University Equestrian Center.

Following each SET, horses were given 2 d of turn out for 90 min of free exercise, 1 d of driving for 15 min without harness or weighted sled, followed by an additional 1 d of turn out for 90 min of free exercise before returning to the specified exercise regimen at the start of the subsequent treatment. The 4 d of light exercise served to permit proper physical recovery from the SET and help to create a smooth transition onto the new ration for the next 28 d period.

Data Collection

Heart rates were recorded at set times throughout all weighted exercise bouts and SET's by utilizing Polar Equine RS800 heart rate monitors (FitMed Health and Fitness, Mill Valley, California). All Polar Equine belts, WearLink W.I.N.D. transmitters, and watches were tested prior to use to insure proper and accurate output. Horses were clipped in the location of electrode placement in order to obtain a clear reading. Electrode belts were dampened and prepared with electrotransmitter gel prior to positioning. Belts and transmitters were placed in the same location on the horse each time. The driver utilized a wrist-worn receiver for the duration of exercise to monitor HR changes. At set times, the driver called out heart rates to a technician for immediate spreadsheet entry.

During the weighted exercise bouts, HR's were obtained and recorded at the end of the 5 min warm-up, the end of the 10 min work out and finally, at the end of the 2 min

cool down. A peak HR and average for the 10 min portion of exercise also were recorded.

Additionally, HR were noted at set times throughout each SET. At time 0, prior to the beginning of the SET, a resting HR was recorded. After the initiation of the SET, heart rates were taken at time 5, 10, 15, 20, 30 and finally at the end of the 10 min cool down. A peak HR for the 30 min SET was documented as well. Following the cool down, the wrist-worn receiver was utilized to transfer HR data onto the Polar ProTrainer Equine Edition computer software for further analysis.

Respiration was recorded at time 0, 30 and after the 10 min cool down. Additionally, temperatures were obtained via rectal thermometer at time 0 and 30.

Venous blood samples were collected via jugular venipuncture for the harvest of serum using blood collection vacutainer tubes without additive. Blood samples were taken at time 0, 30, 1, 6, and 24 h post SET respectively. After obtaining blood, samples were allowed to clot for 1 h prior to being put on ice until refrigeration was available. After all necessary samples were gathered; blood collection tubes were removed from the refrigerator and loaded into the centrifuge, taking care to insure the rotor was balanced. Samples were spun for 24 min at 2200 rpm and 4˚C. After separation, serum was pipeted into 4.5 mL separation tubes, labeled, capped and immediately transported on ice to the Texas A&M Veterinary Medical Diagnostic Laboratory for analysis. Remaining serum from original samples was pipeted into 1.5 mL microcentrifuge tubes, labeled, securely capped, and refrigerated for 24 h before lactate analysis could be

completed. Immediately following completion of lactate analysis, samples were stored in a freezer at -20° C.

Laboratory Analyses

Serum samples were analyzed by the Texas A&M Veterinary Medical Diagnostic Laboratory for analysis. Tests conducted included an Equine Chemistry Panel, LDH, and serum Mg for samples at 0, 6, and 24 h. Serum Mg alone was also analyzed on sample 0.5 h. Further, using a YSI Model 2788 Lactate Analyzer, serum samples 0, 30, and 1 h stored in 1.5 mL microcentrifuge tubes were analyzed for lactate and glucose concentrations in mmol/L.

Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (SAS Institue, version 9.2, Cary, NC, USA). The model will include fixed effects of treatment, sample, and treatment x sample interaction. A *P*-value of $\leq$ 0.05 was considered significant. Furthermore, when significant effects were observed, means were separated by a pair wise t-test.

CHAPTER IV

RESULTS

Magnesium Intake and Potassium Intake

As the experimental design dictated, there was an effect of Trt on average daily intake (ADI) of Mg in both grain and total intakes (g DM) across all horses as a result of differing rations (Table 3). The ADI of Mg in grain alone was significantly different in Trt 1 than in Trt 2, or the low Mg ration ($P<0.001$). Additionally, the mean ADI of Mg in grain was significantly different in Trt 3 than in Trt 2 ($P<0.0001$). However, there was no difference in ADI of Mg in grain between Trt 1 and 3. Although the horses were fed concentrate at 0.70% BW/d throughout the trial, the difference in Mg intake appears to be due to an increase in grain refusals observed in Trt 2 when compared to all others.

Likewise, the ADI of Mg in total was significantly different in Trt 1 when compared to Trt 2 ($P<0.04$). Mean ADI of Mg in total in Trt 3 also was different from Trt 2 across all horses ($P<0.004$). Again, there was no difference on ADI of Mg in total between Trt 1 and 3 ($P>0.05$).

Lastly, there was no significant effect of Trt on ADI of Mg in hay across all horses ($P>0.05$). This would be expected as hay type did not vary throughout the trial.

Table 3. Least squares means for average daily intake (ADI) of Mg in grain, hay, and total (g DM) by treatment across all horses in response to different rations

	Treatment			
Item	1	2	3	SE[1]
ADI (g DM)				
Grain	8.68[a]	6.57[b]	9.52[a]	0.37
Hay	5.51	5.67	5.70	0.26
Total	14.19[a]	12.25[b]	15.22[a]	0.62

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)

There was a significant effect of Trt on ADI of K in both grain and total intakes (g DM) across all horses as a result of differing rations (Table 4). The mean ADI of K in grain alone was different in Trt 1 than in Trt 2 (P<0.001). Additionally, the mean ADI of K in grain was different in Trt 3 than in Trt 2; as was the case in Trt 1, where the mean ADI of K was significantly different than Trt 3 across all horses (P<0.0001).

Likewise, there was a significant effect of Trt on ADI of K in total intake, combining both grain and hay, across all horses in response to different rations (Table 4). The mean ADI of K in total was different in Trt 1 when compared to Trt 3 (P<0.04). Mean ADI of K in total in Trt 2 also was different from Trt 3 (P<0.001). There was no difference on ADI of K in total between Trt 1 and 2 (P>0.05).

Lastly, there was no significant effect of Trt on ADI of K in hay across all horses (P>0.05). This again was to be expected as hay type remained constant during the trial.

Table 4. Least squares means for average daily intake (ADI) of K in grain, hay, and total (g DM) by treatment across all horses in response to different rations

	Treatment			
Item	1	2	3	SE[1]
ADI (g DM)				
Grain	41.84[a]	51.72[b]	23.36[c]	1.74
Hay	72.41	74.63	75.03	3.35
Total	114.25[a]	126.35[a]	98.38[b]	5.04

[1]SE = standard error
[a,b,c]Values in same row not sharing common superscripts differ (P<0.05)

Weekly Exercise

There was no effect of Trt on mean HR across all horses for the weekly exercise sessions (P>0.05). The mean HR's in Trt 1, 2, and 3 were 122.59 ± 3.84, 124.22 ± 2.92, and 125.76 ± 0.68, respectively. The average maximum HR of the horses observed during the weekly exercise likewise was not affected by Trt. However, mean recovery HR for the weekly exercise bouts were affected by Trt, where Trt 1 (77.79 ± 4.23) was higher than Trt 2 or 3 (73.92 ± 3.22, 73.36 ± 5.03). Finally, there was an effect of Trt on the mean total weight in kg pulled by the horses during the week. Treatment 1 (99.39 ± 11.81) was lower than Trt 2 (111.47± 9.44) and furthermore, Trt 2 was lower than Trt 3 (123.57 ± 8.33) at P<0.05.

Mean Heart Rate, Respiration Rate, and Temperature

In response to the SET's on the treadmill, there was no significant effect of Trt on HR at rest, immediately post exercise, or recovery (10 min post exercise) across all horses (P<0.05). Likewise, there was no effect of Trt on maximum HR (P>0.05).

However, there was a significant difference ($P<0.0001$) in HR between sample times within each Trt in response to the SET (Table 5). Furthermore, all fluctuations in mean HR's between sample times were different from each other within each Trt.

Treatment had no effect on RR at rest, immediately post exercise, or recovery across all horses in response to the SET's ($P>0.05$). There was a significant difference ($P<0.01$) in RR between sample times within each Trt throughout the treadmill test (Table 5). Similar to HR's discussed above, RR within each Trt was lowest at rest, amplified immediately post exercise, and followed by a drop at recovery; all changes between sample times were again different from each other within each treatment.

Across all horses, there was a significant effect of Trt on rectal temperature (RT) at rest, as the mean RT at rest in Trt 0 was lower than in Trt 1 or 3 prior to each SET (Table 5). No effect of Trt was observed on RT immediately post exercise on the treadmill ($P<0.05$). However, there was a significant difference in RT between sample times within each Trt across all horses, where resting mean RT was lower than mean RT immediately post SET ($P<0.0001$).

Table 5. Least squares means for heart rate (HR), respiration rate (RR), and rectal temperature (RT) by treatment across all horses in response to SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
HR					
Rest	38.83[c]	49.17[c]	44.67[c]	40.67[c]	5.63
Post[2]	169.17[d]	164.50[d]	171.83[d]	161.00[d]	5.63
Recovery[2]	78.17[e]	75.17[e]	73.33[e]	79.83[e]	5.63
Maximum	172.17	168.17	178.00	167.83	5.63
RR					
Rest	19.33[c]	26.67[c]	24.00[c]	24.67[c]	9.06
Post	121.33[d]	128.00[d]	112.00[d]	118.00[d]	9.06
Recovery	59.50[e]	63.67[e]	61.17[e]	64.00[e]	9.06
RT					
Rest	99.38[ac]	100.72[bc]	100.18[abc]	100.55[bc]	0.39
Post	103.43[d]	103.93[d]	103.73[d]	103.20[d]	0.39

[1]SE = standard error
[2]Post = immediately post SET; Recovery = 10 min following the end of the SET
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d,e]Values in same column under the same item (HR, RR, Temp) not sharing common superscripts differ (P<0.05)

Lactate and Glucose Concentrations

There was no effect of Trt on lactate (LA) concentrations (mmol/L) at any sampling time during the SET's on the treadmill across all horses (P>0.05). Within each Trt however, LA concentrations were significantly different at each sample time. The mean LA concentrations immediately following exercise on the treadmill were consistently higher than those observed at rest or 1 h post exercise (Table 6).

Table 6. Least squares means for lactate (LA) concentrations (mmol/L) by treatment and sample across all horses at rest, immediately post exercise, and 1 h post exercise in response to SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
LA					
Rest	1.08[c]	1.68[c]	1.99[c]	2.12[c]	0.8255
Post	7.89[d]	7.01[d]	7.41[d]	6.30[d]	0.8255
1 h	2.09[c]	1.95[c]	2.29[c]	2.27[c]	0.8255

[1]SE = standard error
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

There was a significant effect of Trt on resting glucose (GLU) concentrations (mmol/L) across all horses as a result of the SET's on the treadmill. Specifically, the mean resting GLU concentration in Trt 2 was lower (P<0.03) than in Trt 0 (Table 7). Furthermore, the mean GLU concentration immediately post exercise on the treadmill was affected by Trt, as the Trt 3 GLU concentration was lower than that observed in Trt 0 (P<0.02). No effect of Trt on GLU concentration 1 h post exercise on the treadmill was observed (P>0.05). However, in Trt 1 the mean GLU concentration at 1 h post exercise was lower than at rest or immediately post SET (Table 7).

Table 7. Least squares means for glucose (GLU) concentrations (mmol/L) by treatment and sample across all horses at rest, immediately post exercise (Post), and 1 h post exercise in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
GLU					
Rest	5.64[a]	5.40[abc]	4.50[b]	4.68[ab]	9.99
Post	6.11[a]	5.76[abc]	5.13[ab]	4.83[b]	9.99
1 h	5.67	5.02[d]	4.96	5.11	9.99

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

Serum Concentrations of Muscle Enzymes

There was an effect of Trt on CK concentrations (U/L) at rest prior to the SET's on the treadmill (Table 8). Mean CK concentration in Trt 0 was significantly lower than that observed in Trt 3 (P<0.03). Otherwise, no effect of Trt on CK concentrations occurred 6 or 24 h post exercise on the treadmill. There was a significant difference between sampling times during the SET in Trt 2 (Table 8). Specifically, in Trt 2, the mean CK concentration 6 h post exercise was higher than at 24 h post exercise (P<0.05). No other differences among sample times on CK concentrations were observed within Trt during the treadmill exercise.

Table 8. Least squares means for creatine phosphokinase (CK) concentrations (U/L) by treatment and sample across all horses at rest, 6 and 24 h post exercise in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
CK					
Rest	189.00[a]	224.67[ab]	244.17[abcd]	306.67[b]	36.96
6 h	250.67	264.33	328.67[c]	283.83	36.96
24 h	220.50	284.50	255.17[cd]	234.00	36.96

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

A significant effect of Trt was noted when comparing the difference in CK concentrations between rest and 24 h post exercise on the treadmill (Table 9). The greatest increase in CK concentration was observed in Trt 1 when compared to Trt 3 (P<0.05).

Table 9. Least squares means for the difference (Diff) in creatine phosphokinase (CK) concentrations (U/L) between rest and 24 h post exercise by treatment across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
CK					
Diff	31.50[ab]	59.83[a]	11.50[ab]	-72.67[b]	33.62

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)

There was no effect of Trt on AST concentrations (U/L) at rest, 6 or 24 h post exercise as a result of the SET's (P>0.05). However, within Trt 0, the AST concentration 24 h post exercise was significantly lower than at 6 h post exercise on the treadmill (Table 10). Additionally, within Trt 3, the mean AST concentration 24 h post exercise was lower than that observed at either rest or 6 h post SET.

Table 10. Least squares means for aspartate transaminase (AST) concentrations (U/L) by treatment and sample at rest, 6 and 24 h post exercise across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
AST					
Rest	263.67[cd]	269.67	270.33	270.17[c]	11.93
6 h	265.17[c]	275.50	263.17	274.83[c]	11.93
24 h	248.17[d]	268.00	265.17	255.00[d]	11.93

[1]SE = standard error
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

There was no effect of Trt on LDH concentrations (IU/L) at rest, immediately post exercise, or 6 h post exercise as a result of the SET (P>0.05). However, within Trt 0, the mean LDH concentration 6 h post exercise was significantly different than at 24 h post exercise (Table 11). Likewise, within Trt 3, mean LDH concentration 6 h post exercise also was higher than at 24 h post exercise on the treadmill (P<0.005).

Table 11. Least squares means for lactate dehydrogenase (LDH) concentrations (IU/L) by treatment and sample at rest, immediately post exercise (Post), and 6 h post exercise across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
LDH					
Rest	282.17[cd]	299.18	312.55	298.32[cd]	35.01
6 h	309.80[c]	321.92	314.22	300.08[c]	35.01
24 h	288.57[d]	336.92	295.82	269.32[d]	35.01

[1]SE = standard error
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

When comparing the difference in LDH concentration between rest and 24 h post exercise on the treadmill, there was a significant effect of Trt across all horses (P<0.05). The difference in LDH concentration in Trt 1 was greater than that observed in either Trt 2 or 3 (Table 12).

Table 12. Least squares means for the difference (Diff) in lactate dehydrogenase (LDH) concentrations (IU/L) between rest and 24 h post exercise by treatment across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
LDH					
Diff	14.00[ab]	37.67[a]	-16.83[b]	-29.00[b]	15.66

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)

There was an effect of Trt on alkaline phosphatase (ALP) concentration (U/L) at rest and 6 h post exercise as a result of the SET's on the treadmill ($P<0.05$). The resting ALP concentration in Trt 2 was lower than in Trt 3 (Table 13). Additionally, the same held true for the ALP concentration 6 h post exercise, as Trt 2 was lower than Trt 3 across all horses following the SET. Within treatments, ALP concentration differed significantly between sample times (Table 13). Mean ALP concentration 6 h post exercise was higher ($P<0.05$) than 24 h post exercise in both Trt 0 and 3 due to the treadmill test.

Table 13. Least squares means for alkaline phosphatase (ALP) concentrations (U/L) by treatment and sample at rest, 6 and 24 h post exercise across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
ALP					
Rest	129.00[abcd]	118.67[ab]	117.67[a]	147.83[bcd]	10.53
6 h	132.83[abc]	121.50[ab]	116.67[a]	148.00[bc]	10.53
24 h	127.00[d]	120.33	119.17	141.83[d]	10.53

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ ($P<0.05$)
[c,d]Values in same column not sharing common superscripts differ ($P<0.05$)

Phosphorus and Calcium Concentrations

A significant effect of Trt on serum phosphorus (P) concentrations (mg/dl) at rest and 6 h post exercise was observed in response to the SET's ($P<0.05$). The mean serum P concentration in Trt 0 at rest was significantly lower than in Trt 1, 2, or 3 (Table 14).

Additionally, 6 h post exercise on the treadmill, the mean serum P concentration in Trt 3 was higher than that observed in treatment 0, 1, or 2 across horses (P<0.05). Within each Trt, serum P concentration varied significantly at rest, 6 and 24 h post exercise Table 14). Specifically, in Trt 0, the resting serum P concentration was lower than at 6 or 24 h post exercise test (P<0.05). Furthermore in Trt's 1 and 2, the mean serum P concentration 24 h post exercise was significantly lower than at rest or 6 h post exercise on the treadmill across all horses (P<0.05). Finally, the mean serum P concentration 6 h post exercise in Trt 3 was significantly higher than at rest or 24 h post SET (P<0.05).

Table 14. Least squares means for serum phosphorus (P) concentrations (mg/dl) by treatment and sample at rest, 6 and 24 h post exercise across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
P					
Rest	2.22[ac]	3.30[bc]	3.43[bc]	3.51[bc]	0.32
6 h	2.94[ad]	3.75[ac]	3.80[ac]	4.24[bd]	0.32
24 h	2.91[d]	3.17[d]	3.26[d]	3.42[c]	0.32

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

There was a significant effect of Trt on the difference in serum P concentration between rest and 24 h post exercise test on the treadmill (Table 15). The mean difference serum P concentration in Trt 0 was greater than Trt 1, 2, or 3 across all horses (P<0.05).

Table 15. Least squares means for the difference (Diff) in serum phosphorus (P) concentrations (mg/dl) between rest and 24 h post exercise by treatment across all horses in response to SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
P					
Diff	0.69[a]	-0.15[b]	-0.17[b]	-0.09[b]	0.24

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)

There was a significant effect of Trt on serum Ca concentration (mg/dl) at rest across all horses as a result of the SET's (Table 16). The mean serum Ca concentration in Trt 3 was significantly higher than in Trt 0, 1, or 2 (P<0.05). However, there was no effect of Trt on mean serum Ca concentration 6 or 24 h post exercise on the treadmill (P>0.05). Within Trt, the resting mean serum Ca concentration was lower than at 6 or 24 h post exercise across all horses (Table 16). There was no significant difference between resting, 6 or 24 h post exercise serum Ca concentrations due to the SET's in Trt 1, 2, or 3 (P>0.05).

Table 16. Least squares means for serum calcium (Ca) concentrations (mg/dl) by treatment and sample at rest, 6 and 24 h post exercise across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
Ca					
Rest	10.70[ac]	11.88[a]	12.23[a]	12.57[b]	0.63
6 h	12.32[d]	11.82	11.98	12.23	0.63
24 h	12.65[d]	12.12	12.05	12.47	0.63

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d]Values in same column not sharing common superscripts differ (P<0.05

Serum Magnesium Concentrations

There was a significant effect of Trt on serum Mg concentrations (mEq/L) at rest, immediately post exercise, and 24 h post exercise across all horses as a result of the SET's on the treadmill (Table 17). Mean serum Mg concentrations were significantly higher in Trt 3 than all other treatments at resting, immediately post exercise, and 24 h post exercise on the treadmill (P<0.05). Serum Mg concentration 6 h post exercise however, was not significantly affected by Trt (P>0.05). Within Trt's, mean serum Mg concentrations varied significantly at sample times during the SET's (Table 17). In Trt 1, 2, and 3, mean serum Mg concentrations 6 h post exercise were significantly lower than at rest, immediately post exercise, or 24 h post exercise on the treadmill (P<0.05).

Table 17. Least squares means for serum magnesium (Mg) concentrations (mEq/L) by treatment and sample at rest, immediately post exercise (Post), 6 and 24 h post exercise across all horses in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
Mg					
Rest	1.63[a]	1.79[ac]	1.77[ac]	2.07[bc]	0.09
Post	1.62[a]	1.73[ac]	1.75[ac]	2.02[bc]	0.09
6 h	1.56	1.61[d]	1.58[d]	1.71[d]	0.09
24 h	1.57[a]	1.78[ac]	1.80[ac]	2.07[bc]	0.09

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d]Values in same column not sharing common superscripts differ (P<0.05)

CHAPTER V

DISCUSSION

Exertional rhabdomyolysis has been recognized for more than 100 years in horses as a syndrome of exercise related muscle pain and cramping (Valberg et al., 1999b). Although ER comprises several myopathies, each varying in pathogenesis, universal symptoms typically include elevated muscle enzyme concentrations, visual signs of stiffness, cramping and excessive sweating. In the present study, no observations of cramping or muscle stiffness were noted in the horses during any of the SET's. Furthermore, serum muscle enzyme concentrations appeared to change insignificantly and thus, could not allow for confident diagnosis of the presence of any type of ER. Regardless, some interesting findings were observed with regard to dietary Mg and exercise, and will further be discussed below.

Magnesium Intake and Potassium Intake

Total ADI of Mg was lowest in Trt 2, the low Mg ration, when compared to Trt 1 and 2. This finding is consistent with initial expectations that ADI of Mg would be lowest in the low Mg diet and highest in Trt 3, the high Mg diet. Interestingly, upon subjective observation, grain refusals appeared to be higher in Trt 2 compared to all others. This may have been indicative of additional dietary factors, for example, palatability, or digestive upset, resulting from a high K or low Mg diet, which may have played a role in decreased intake. The ADI of K in grain was highest in Trt 2, where K was increased in an attempt to reduce the digestibility of Mg, thus helping to create a Mg

deficient status. Furthermore, total ADI of K intake also was higher in Trt 2 when compared to Trt 3.

Mean Heart Rate, Respiration Rate, and Temperature

Neither HR nor RR during the SET's was affected by Trt across all of the horses. The intent of the weekly exercise protocol through all 3 treatments was to maintain an even level of fitness across all horses. Had the level of fitness increased, it would be assumed that HR's and RR's would gradually decrease over the 105 d experiment as each horse's conditioning increased. However, prior studies in horses have determined that increased training fails to cause any changes in HR (Foreman, 1984; Evans and Rose, 1988). In the present trial this appeared to be the case as even the mean maximum HR during the SET's remained unchanged over all 3 treatments.

Within each respective Trt, HR and RR varied significantly as would be expected during any exercise bout. Heart rate is known to increase linearly with exercise intensity in order to maintain cardiac output and blood pressure (Brooks et al., 2005). Similarly, RR increases during exercise because tidal volume and frequency of breathing both increase, up to three-fold resting levels (Brooks et al., 2005). In the present study, both HR and RR were lowest at rest, significantly elevated by the end of exercise and subsequently decreased upon the completion of a 10 min recovery period.

There was an effect of Trt on resting RT as the mean in Trt 0 (99.38±0.34) was significantly lower than Trt 1, or 3 (100.72±0.51, 100.55±0.15). The likely cause of elevated resting temperatures during the last 3 SET's can be attributed to increasing

ambient temperatures that occurred with changes in the seasons. Although all SET's were run at the same time of day in an attempt to combat changes in ambient temperatures, the fluctuations in humidity paired with other seasonal changes were uncontrollable variables that may have influenced resting rectal temperatures. Additionally, RT significantly increased during the SET within each Trt. As previously documented, temperatures elevate with the onset of exercise and magnitude is dependent upon the degree of activity more so than by the external environment (Neilsen, 1938). At the beginning of exercise, heat accumulates within the working muscles until muscle blood flow reaches the level of demand at which point, heat is then transferred to the body core as well as the skin for heat dissipation via sweat (Thiel et al., 1987).

Lactate and Glucose Concentrations

Treatment had no effect on LA concentrations at rest, immediately post exercise, or 1 h post exercise. Similar findings were reported by Thornton et al. (1983), where training did not significantly affect resting LA concentrations. Additionally, Hinchliff et al. (2002), observed no effect of training on LA concentrations immediately following a high intensity exercise bout. Therefore, it can be suggested that conditioning did not influence LA concentrations at any sample time during the present study.

There obviously was a difference between LA concentrations due to the SET within each Trt, where those at rest and 1 h post exercise on the treadmill were significantly lower than immediately after exercise. This was to be expected and is comparable to results reported by Brady et al. (1977), in which blood lactate

concentrations were highest in horses immediately after exercise. Additionally, although not significant, mean LA did begin to fall to baseline levels by the 1 h sampling allowing for some comparison with previous research supporting lactate levels return to pre-exercise levels by 24 h (Milne et al., 1976; Brady et al., 1977). Overall, increased LA concentration immediately post exercise was indicative of the presence of anaerobic metabolism, as 4.0 mmol/L is the approximate point at which the transition from aerobic to anaerobic metabolism takes place. The LA concentrations immediately following exercise ranged from 6.30±0.8255 to 7.89±0.8255 mmol/L, yet were 3 times lower than some values that have been observed in other intense exercise studies (Harris and Snow, 1988; Schuback and Essen-Gustavsson, 1998). The intensity of the SET administered in each Trt may not have been great enough to illicit the changes originally expected. Alternate studies however, have reported a lack of elevated LA concentrations in horses exhibiting a form of ER following submaximal exercise (MacLeay et al., 2000).

Glucose concentrations at rest were significantly affected by treatment, as Trt 2 (4.50±0.3630 mmol/L) was lower than 0 or 1 (5.64 and 5.40±0.3630). This potentially could be due to 1 of 2 causes. First, in certain situations, excitation or early stages of exercise can stimulate an increase in GLU due simply to release of adrenalin (Marcella, 2009). In the present study, the first 2 SET's may have increased GLU through the process of fitting the HR monitors and being loaded onto the treadmill, which may have caused excitation of the horses since it was a new experience. However, if this were the case, it would be expected that GLU concentrations would consistently decrease at rest as the horses became more accustomed to the SET process and this was not apparent.

Therefore, it may be fair to consider the low Mg ration as the potential cause of lower GLU concentrations in Trt 2. As stated previously, Mg plays a primary role in cellular energy production (Newhouse and Finstad, 2000; Rude, 2000). A low Mg diet may attribute to a disruption in mitochondrial metabolism and further, a decrease in energy metabolism as documented in ruminants (Fontenot et al., 1989). If this were the case, it would support the findings of lower GLU concentrations and possibly, decreased glycogen storage in the muscle, at rest in Trt 2.

Glucose concentrations exhibited small increases and decreases during the SET's, and although they only appeared significant in Trt 1, these minor fluctuations are in agreement with previous findings in horses during exercise (Hambleton et al., 1980). Typically, low blood GLU after 3 to 4 h of prolonged exercise is associated with fatigue in the horse (Carlson et al., 1965; Lindholm and Piehl, 1974; Lindholm and Saltin, 1974). In the present study, GLU concentrations were not analyzed at 3 or 4 h. However, GLU rose immediately post SET and decreased by 1 h post treadmill test. This was demonstrated in Trt 0, 1, and 2, although in Trt 3, GLU concentrations 1 h post SET were higher than resting, or concentrations immediately post exercise. These observations may be attributed to the increased levels of Mg in the diet. Lukaski et al., 1983, suggests ionic Mg may facilitate oxygen delivery to working muscles in trained athletes and furthermore, a review of this and multiple other studies determined a positive correlation between plasma Mg and VO_2 max (Bohl and Volpe, 2002). If this is the case, an increase in Mg would be expected to enhance the facilitation of oxygen delivery to exercising muscle. Therefore, a horse would have the ability to perform

longer without experiencing significant decreases in GLU concentrations, or becoming fatigued. The current findings in this study may be supported by this theory. However, the decrease in the depletion of GLU concentrations across the duration of the trial may also have been influenced by an increased level of conditioning in the animal.

Serum Concentrations of Muscle Enzymes

The only visible effect of Trt on CK activity occurred at rest, where the mean CK concentration in Trt 0 (189.00) was significantly lower than in Trt 3 (306.67). It is not clear as to why this difference occurred. If there was an increase in conditioning, it would be expected that the CK concentrations prior to exercise would decrease over the course of the study and that appears not to be the case. MacLeay et al. (2000) observed increases in CK prior to exercise in RER horses fed a high-grain diet when compared to a high fat diet. In the present study, the diets in Trt 1, 2, and 3 contained fat percentage levels twice that of the diet fed prior to the beginning of the trial. It could be speculated a lower fat diet may have contributed to the higher CK concentrations in Trt 3, although the difference in percent fat was minimal between treatments. Therefore, it is also important to recognize that none of the horses used in this study were previously diagnosed with RER, which may have influenced the responses observed. Regardless, increases in resting CK concentration in Trt remain unexplained.

Typically, CK activity peaks within 4 to 6 h post exercise and may be up to 4 times the normal values (MacLeay et al., 2000). A peak in CK was observed at 6 h in Trt 2, however, drastic elevations in CK concentrations were not observed at these

sample times as would be expected following the SET. As such, the only significant change in CK activity was seen in Trt 2, where the mean CK concentration 6 h following the treadmill was greater than 24 h post exercise (328.67±36.96 and 255.17±36.96). This could be explained by the low Mg ration which may have resulted in decreased membrane stability within the muscle cells, subsequently causing an increased leak of CK into the blood (Harris et al., 1998). Furthermore, when analyzing the change in CK concentration from rest to 24 h post SET, there was a significant Trt effect. The change in Trt 3 was lowest when compared to Trt 0, 1, and 2. This result may be diet influenced as the ration in Trt 3 contained the most Mg. However, in general the low magnitude of the changes in CK concentrations observed over the course of the study suggest the SET protocol may not have been strenuous enough to illicit the magnitude of increases in CK activity indicative of muscle damage as initially proposed (Anderson, 1975; Milne et al., 1976; Ross et al., 1983).

No effect of Trt was observed on AST concentrations at any sampling time during the SET's. Classically, AST peaks at 24 h post exercise and may remain elevated for several days (Snow and Valberg, 1994; Harris et al., 1998). However, in the present study the mean AST concentration 24 h post treadmill test in both Trt 0 and Trt 3 was significantly lower than at 6 h post exercise. This contrast in time of peak AST activity in the present study when compared to others may be due to slow peaking AST concentrations, where a 36 h sample would have been more useful. Additionally, as previously mentioned, lack of significant changes in AST activity may be attributed to the failure of the SET's to be strenuous enough to illicit changes in the muscle enzymes

or cellular membrane permeability (Harris et al., 1998). Finally, it is apparent that dietary Mg had no effect on AST concentrations throughout the SET's in this study.

There also was no significant effect of Trt on LDH concentrations in the present trial. Since LDH activity characteristically represents intense exercise and can be paired with AST (Marcella, 2009), it is probable that with no effect of Trt, the SET was not intense enough to induce changes. Further, if elevations of LDH concentrations had occurred, indicating muscle dysfunction, it would have done so 24 h post exercise (Marcella, 2009). In the present study no significant elevations were noted 24 h post SET. Furthermore, in Trt 0 and 3, the 24 h concentrations were lower than those at rest or 6 h post exercise.

Similar to changes over sample times in CK concentrations, the change in LDH concentrations from rest to 24 h post exercise, within Trt 2 and Trt 3 were significantly lower than in Trt 1. The only explanation for this observation appears to be related to level of conditioning, since Trt 2 represented a low Mg ration and Trt 3, a high Mg ration. It appeared that diet did not influence the changes noted in LDH concentrations.

An effect of Trt was observed on ALP concentrations at rest and 6 h post SET. At both sampling times, ALP was significantly lower in Trt 2 than in Trt 3. Classically, ALP trends follow trends in CK and AST concentrations, whereby, increases in activity result from an intense exercise bout and probable muscle damage. However, in the present study the lack of observed increases in CK or AST, along with no significant correlations, cause speculation for an alternative explanation outside of muscle breakdown. It has been suggested that reduced levels of ALP may signify a Mg

deficient status. The lower ALP concentrations in Trt 2 at rest and 6 h post SET compared to Trt 3 are supported by this theory since Trt 2 was the low Mg diet. Studies in rats, cattle, and mice have repeatedly shown marked decreases in ALP when in a Mg deficient state (Larvor et al., 1964; Heaton, 1965; Hamuro, 1971; Robeson et al., 1979). This confirms that Trt 2 may have actually induced somewhat of a Mg deficient state in the horses as intended. Furthermore, ALP concentrations were significantly higher in Trt 3 than Trt 2 when Mg was supplemented in the diet, suggesting the increase in Mg influenced the increased ALP activity, although this has not been documented in previous studies.

Serum Phosphorus and Calcium Concentrations

Treatment had a significant effect on resting serum P concentrations as well as concentrations 6 h post SET. The mean P concentration at rest was significantly lower in Trt 0 than in any of the other treatments. Furthermore, the 6 h post exercise serum P concentrations were lowest in Trt 0 as well. According to Viana et al. (2007), P exhibits a diurnal rhythm in sedentary horses, or those just walking. However, this rhythm is masked upon the inclusion of exercise and is believed to be due to increased P losses in the sweat and feces as observed by Schryver et al. (1978) and Hoyt et al. (1995). These findings would support the low P concentrations in Trt 0, as the horses were assumed to be untrained and therefore would most likely have experienced the highest P losses when compared to the subsequent treatments. While the difference in P concentrations across treatments likely can't be explained solely due to losses through feces and sweat, since

the ambient temperatures increased over the course of the study, another factor must be considered. Although controversial, two studies have indicated an increased retention of P in horses undergoing exercise when compared to those that were sedentary (Young et al., 1989; Elmore-Smith et al., 1999). This would support the findings in the present study as P concentrations across all sample times, although not significant, tended to be highest in Trt 3, a point at which the horses had undergone exercise for the entire trial, as opposed to Trt 0 where horses had not previously been subjected to any type of exercise. Furthermore, considering only dietary effects, similar findings of increased P concentrations have been documented in cattle and sheep where serum P concentrations were observed to be highest with increased levels of dietary Mg (Care, 1960; Chester-Jones et al., 1989, 1990). This could also be a possible reason for the results obtained in the current study as Trt 3 presented the highest levels of dietary Mg. To accurately determine whether the cause of increases in P concentrations 6 h post exercise, were due to diet or the SET, serum Mg and urinary Mg would have to be analyzed immediately following and 1 h post exercise.

Similar to observed trends in CK and LDH activity, the change in P concentration from rest to 24 h post SET was numerically lowest in Trt 3, although only significantly different from Trt 0. This would again be supported by the idea that exercise resulted in increased P retention, thus a minimal change from rest to 24 h post SET, or possibly that the increased dietary Mg was the causative factor.

Mean serum Ca concentrations failed to follow a distinct pattern across sample times in each Trt. However, the resting Ca concentration in Trt 3 was higher than those

observed in Trt 0, 1, or 2. This may be in part due to the ration in Trt 3 containing the highest level of Ca (1.52% DM) compared to Trt 0 and 1 (0.76% DM), or Trt 2 (1.50% DM), respectively. Additionally, the resting P concentrations were affected by Trt in a comparable fashion to Ca, further indicating a proportional fluctuation of each as would be expected with an adequate Ca:P ratio.

Serum Magnesium Concentrations

A significant effect of treatment on mean serum Mg was observed at rest, immediately post and 24 h post exercise on the treadmill. Previous research indicates that exercise causes a redistribution of Mg in the body and the type of exercise and present Mg status both may influence this redistribution (Laires and Monteiro, 2001; Lukaski, 2001). While a review of conflicting studies as to whether or not changes in total intracellular Mg are indicative of changes in total serum Mg concentrations, it is confirmed in humans, horses, and rats that intercompartmental shifts of Mg occur upon induction of intense exercise in order to meet immediate metabolic needs (Nielsen and Lukaski, 2006). Therefore, it is probable the increases in serum Mg at rest, immediately post and 24 h post exercise in Trt 3 over all other treatments, were a result of an increased Mg status in the body, possibly paired to a decrease in immediate metabolic needs of the horse as conditioning increased over the study. Additionally, the increase in serum Mg at rest observed across treatments was negatively correlated to HR immediately post exercise ($r = -0.43$), further suggesting an effect of dietary Mg on the metabolic needs of the horse; as Mg concentration at rest increased, HR at the end of the treadmill test decreased. With the role Mg plays in energy metabolism, it could be

suggested that a decrease in Mg status may cause an increase in HR throughout exercise as the demands on the body become greater. A low Mg status would influence the rate at which a horse reached fatigue by decreasing efficiency of energy production. Additionally, since HR dramatically increases just prior to reaching the point of fatigue, Mg could affect the rate at which a horse's HR increases, and further, becomes fatigued. However, it is important to consider conditioning as another effect that may have contributed to the lowered HR's immediately post exercise. This also would be a probable conclusion as the decreased HR's were noted towards the end of the trial, in Trt 3, where horses would most likely have been in the greatest condition as well as being provided with a high Mg ration.

Within Trt 1, 2, and 3, serum Mg 6 h post exercise on the treadmill was significantly lower than all other sample times. It has been previously established that a significant portion of Mg is lost via sweat when exercise takes place in hot and humid conditions (Consolazio, 1963), as well as Mg lost in urine post-exercise; both of which are documented to be further amplified by a strenuous work out (Bohl and Volpe, 2002). For racehorses in training, long term strenuous exercise is said to be the predominant cause of increased urinary excretion of Mg as opposed to increased intestinal absorption efficiency (Stephens et al., 2004). While the present study did not utilize what would not be considered long-term strenuous exercise, in humans it is postulated the exercise-induced increase in Mg excretion depends on not only intensity of exercise, but also the relative contribution of anaerobic metabolism to the total energy expenditure during exercise (Deuster et al., 1987). Accordingly, Nielsen and Lukaski (2006) suggest that

amplified urinary excretion of Mg post-exercise may be due to circulating lactic acid concentrations. Rises in blood lactic acid may elevate plasma phosphorous and thus, metabolic acidosis which has been shown to cause magnesuria via reduction of tubular reabsoption of Mg (Bohl and Volpe, 2002).

Without knowing intracellular Mg concentrations, it is reasonable to speculate that the large decrease in mean serum Mg at 6 h within each Trt was a result of serum Mg losses via sweat and urine either as a result of intense exercise or increased anaerobic metabolism in response to the SET's. However, a portion of the loss in serum Mg may have resulted from the compartmental shift of Mg within the body into intracellular spaces. In humans, serum Mg levels typically return to baseline within 24 h which also was observed in this study (Bohl and Volpe, 2002).

The greatest shift in serum Mg was observed in Trt 3 at the 6 h sample. This value is representative of a 15 percent decrease in serum Mg immediately following exercise to 6 h post; subsequently followed by a 17 percent increase back up to baseline value at 24 h. Similar results were observed in humans on a high Mg diet where changes in plasma Mg induced by exercise were 13 times greater than changes seen in the same athletes post-exercise prior to being placed on the high Mg diet (Westmoreland et al., 2004). Furthermore, as suggested by Nielsen and Lukaski, 2006, small increases or decreases in plasma Mg with exercise may indicate a deficient Mg status, whereas large changes most likely indicate, at minimum, a normal Mg status during exercise. This theory is applicable to the present study as the large changes in serum Mg occurred during Trt 3, or the high Mg diet. Finally, mean serum Mg in Trt 3 recovered to baseline

values by 24 h. Such recoveries have been observed in cross country ski racers who experienced a 10 percent decrease in serum Mg post-race, whereby at 24 h their serum Mg had rebounded back up to baseline values (Refsum et al., 1973).

When only considering the dietary effects on serum Mg concentrations, the results obtained in this study suggest a diet containing increased Mg (Trt 3) causes an overall increase in serum Mg concentration as was observed at rest, immediately post and 24 h post SET. Similar findings have been documented in both steers and sheep, where increases in the dietary intake of Mg resulted in significant elevations of serum Mg concentrations (Gentry et al., 1978; Chester-Jones et al., 1989, 1990). However, in one of the reviewed studies, it took cattle an average of 105 d on rations supplemented with varying levels of Mg to exhibit notable increases in serum Mg concentrations (Chester-Jones et al., 1990). The observed increase in serum Mg concentrations in the present study occurred after only a 28 d period. Although not observed in this trial, rats fed a Mg deficient diet revealed decreased serum Mg concentrations after just 14 d (Robeson et al., 1979). In this study, the high Mg diet in Trt 3 influenced the change in serum Mg concentrations at rest, immediately post and 24 h post SET as observed in this study.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Muscle disorders are highly prevalent in the performance horse industry. Research surrounding a variety of muscle myopathies remains diverse as investigations focus on the underlying causes, prevalence within breed-types, and effectiveness of management options. However, the specific causal pathogenesis of exercise induced ER episodes has yet to be elucidated. Therefore, further exploration into all aspects of ER is warranted. It has long been documented in humans that Mg plays an essential role in muscle function and has been suggested that it may have the ability to influence athletic performance. Due to the lack of investigation in the equine model, the objective of this study was to evaluate the effects of varying levels of dietary Mg on muscle contractile properties in exercising horses.

None of the horses used in this project were diagnosed with ER prior to the study. Furthermore, none exhibited drastic increases in any of the serum muscle enzymes that classically are used to determine muscle damage or breakdown. It is apparent that the SET on the treadmill was not intense enough to induce significantly elevated concentrations of CK, LDH, AST, and ALP. In order to insure the SET's from treatment to treatment were comparable, the same protocol was used throughout the trial. However, it is assumed that the SET's were affected to some extent by training even though there was an attempt to keep all horses at a relative level of fitness. Likewise, due to the conditions of this study, period and treatment were confounding and therefore,

no claims can be made with confidence that the observed results were due solely to a training effect or solely to an effect of diet.

Regardless, analysis of the data collected suggests dietary Mg does influence the serum Mg concentration in horses. A high Mg ration notably increased the resting serum Mg concentrations, as well as those immediately post exercise and 24 h following the SET. It can also be speculated, as previously documented, lowered serum ALP concentrations can indicate a low, if not deficient Mg status in horses. Finally, serum Mg concentrations at rest appeared to be negatively correlated with HR immediately post exercise, suggesting perhaps that dietary Mg paired with an increased level of fitness may be important factors that influence exercise performance.

While no symptoms of ER were observed in this study, nor significant effect of Trt on serum muscle enzymes outside of ALP, this trial provides valuable preliminary data for future research in this area of interest. Additional investigation is necessary to further explore the effects of dietary Mg on muscle function in the equine model, as the benefits could be immense. Any improvement or greater knowledge regarding muscle disorders in performance horses could not only provide insight, but more importantly, could aid in enhancing performance ability via increased efficiency of muscle contraction. This would be beneficial as it has the potential to effectively increase the longevity and profitability of the equine athlete.

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APPENDIX

Table 1. Weights (kg) recorded for each horse by treatment (Trt 1, Trt 2, and Trt 3) and week (0-11)

	Trt 1				Trt 2				Trt 3			
	Week											
Item	0	1	2	3	4	5	6	7	8	9	10	11
Chance	509.55	520.45	525.00	529.09	537.27	540.45	542.27	545.00	553.18	549.55	552.27	560.00
Daisy	390.00	408.64	408.18	417.27	414.55	421.36	418.64	426.82	429.55	425.00	425.91	422.73
Frenchy	432.27	447.73	436.82	437.27	439.09	444.55	445.45	442.27	443.18	447.73	438.64	444.09
Juno	405.00	410.45	414.55	425.00	424.55	426.82	432.27	433.18	434.09	433.48	432.27	433.64
Sahara	485.00	496.36	508.18	518.64	513.64	521.82	521.82	518.64	516.82	520.45	518.18	529.09
Pizzaz	484.09	495.00	491.36	503.18	502.73	511.82	513.18	517.73	515.45	515.45	514.55	515.45

Table 2. Least squares means for average HR (AVG HR), maximum HR (MAX HR), recovery HR (REC HR), and weight pulled (WT) in kg across all horses by treatment for weekly exercise sessions

	Treatment			
Item	1	2	3	SE[1]
AVG HR	122.59	124.22	125.76	0.94
MAX HR	152.69	152.58	155.62	1.77
REC HR	77.79[a]	73.92[b]	73.36[b]	1.06
WT	99.39[a]	111.47[b]	123.57[c]	3.16

[1]SE = standard error
[a,b,c]Values in same row not sharing common superscripts differ (P<0.05)

Table 3. Least squares means for creatine (CRE) concentrations (mg/dl), globulin (GLOB) concentrations (g/dl), and gamma glutamyltransferase (GGT) concentrations (U/L) by treatment and sample at rest, 6 and 24 h post exercise in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
CRE (mg/dl)					
Rest	1.77[a]	1.42[a]	1.48[bc]	1.72[a]	6.32
6 h	1.89	1.39	1.58[d]	1.70	6.32
24 h	1.59	1.05	1.56[d]	1.68	6.32
GLOB (g/dl)					
Rest	2.50	2.83	2.97	2.83[cd]	0.27
6 h	2.75	2.82	2.82	3.45[d]	0.27
24 h	2.77	2.73	2.87	2.65[c]	0.27
GGT (U/L)					
Rest	14.17	12.83	13.33	13.63[ab]	1.25
6 h	13.83	13.67	13.17	13.57[a]	1.25
24 h	12.83	14.17	12.83	11.68[b]	1.25

[1]SE = standard error
[a,b]Values in same row not sharing common superscripts differ (P<0.05)
[c,d]Values in same column, within item, not sharing common superscripts differ (P<0.05)

Table 4. Least squares means for blood urea nitrogen (BUN), direct bilirubin (DB), and total bilirubin (TB) concentrations (mg/dl), albumin (ALB) and total serum protein (TSP) concentrations (g/dl) by treatment and sample at rest, 6 and 24 h post exercise in response to the SET's on the treadmill

	Treatment				
Item	0	1	2	3	SE[1]
BUN (mg/dl)					
Rest	17.18	16.32	15.67	17.08	0.91
6 h	17.95	16.75	16.52	16.42	0.91
24 h	17.33	17.43	16.65	16.75	0.91
DB (mg/dl)					
Rest	0.22[a]	0.32[b]	0.23[a]	0.19[a]	0.03
6 h	0.23[a]	0.32[b]	0.22[a]	0.20[a]	0.03
24 h	0.21[a]	0.31[b]	0.19[a]	0.20[a]	0.03
TB (mg/dl)					
Rest	1.47[acd]	1.30[ab]	1.10[ab]	1.04[b]	0.15
6 h	1.46[c]	1.28	1.17	1.05	0.15
24 h	1.20[d]	1.17	1.22	0.97	0.15
ALB (g/dl)					
Rest	3.57[c]	3.53	3.62	3.62	0.09
6 h	3.57[c]	3.56	3.50	3.53	0.09
24 h	3.33[d]	3.45	3.53	3.43	0.09
TSP (g/dl)					
Rest	6.07	6.37	6.58	6.45[cd]	0.27
6 h	6.32	6.37	6.32	6.98[c]	0.27
24 h	6.10	6.18	6.40	6.08[d]	0.27

[1]SE = standard error

[a,b]Values in same row not sharing common superscripts differ ($P<0.05$)

[c,d]Values in same column, within an item, not sharing common superscripts differ ($P<0.05$)

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