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CÂMPUS DE JABOTICABAL

EFFECT OF DIETS WITH VARYING STARCH CONTENT ON MUSCLE GLYCOGEN CONCENTRATIONS DURING TRAINING AND REPLENISHMENT AFTER HIGH-INTENSITY EXERCISE

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Zootecnista

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AUTHOR'S CURRICULUM VITAE

Vanesa Silva de Mesquita – Born February 2nd, 1974, in Santos-SP, identification number 21319710-8 SSP/SP. Graduated in Animal Science at Universidade Estadual Paulista – Faculdade de Medicina Veterinária e Zootecnia – Campus Botucatu in 1997. After graduating, worked as assistant trainer at an Arabian horse farm in Nevada, United States. In 1999, started work as field technician in equine nutrition for a major Brazilian feed company. In 2003 took over responsibility for the equine product line in the same company, function in which remained until 2006. In this same year, became partner at Equimix Ltd., a company exclusively dedicated to manufacturing and selling equine feeds and supplements, position held until the present day.

"Is it the smell of their body as I hug their long neck,
or the scent only a horse has that I can't forget?
Is it the depth of their eyes as they contentedly rest?
No, it's just being around them that I like the best."
Teresa Becker

To my parents, who always supported all my decisions,

no matter how crazy they seemed.

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SUMMARY

EFFECT OF DIETS WITH DIFFERENT STARCH CONTENT ON GLYCOGEN LEVELS DURING TRAINING AND REPLENISHMENT AFTER HIGH-INTENSITY EXERCISE

ABSTRACT- Muscle glycogen is an important energy substrate and a potentially limiting factor of performance in horses. Due to the large number of studies using low-starch, high-fat and fiber concentrates for the management of metabolic diseases, the use of such feeds has become widely popular. However, there is scarce evidence of the effectiveness of such feeds on glycogen maintenance and replenishment. To evaluate the effect of three diets varying in starch content on the maintenance of glycogen levels during a training period and on repletion of glycogen stores after high-intensity exercise, six previously conditioned Thoroughbred horses were used in a 3 x 3 Latin Square design. Horses were fed (at 1kg/100kg BW/day) either a high-starch (HS), a moderately starch-rich, high-fat concentrate (MS) or a low-starch, high-fat and fiber concentrate (LS). Forage was fed at 1.25 kg/100 kg BW/day in all treatments. Horses were trained for three weeks and then underwent three days of strenuous exercise designed to substantially deplete glycogen reserves, and were subsequently observed over four days of recovery. Muscle biopsies were obtained before depletion and at 0, 24, 48 and 72 hours post-depletion. Day one of depletion was an incremental exercise test (IET) and blood was sampled at each speed step for plasma glucose and lactate. During the IET horses wore a loose-fit mask for assessment of Oxygen consumption (VO_2), Carbon Dioxyde production (VCO_2) and Respiratory Exchange Ratio (RER). A submaximal exercise test was performed both before and after depletion of glycogen reserves to verify alterations in energy substrate utilization due to depletion. Plasma glucose, lactate and RER during the IET were not different among treatments. RER during the submaximal exercise test were lower for the LS treatment ($P<0.05$). Post-depletion RER for HS and MS were different from pre-depletion RER in the submaximal test ($p<0.05$). Muscle glycogen repletion was lower for the LS treatment after 72 hours of recovery ($P<0.05$). Low-starch concentrates seem to be inefficient in replenishing glycogen

stores after high-intensity exercise bouts, although they are able to maintain glycogen levels during a regular training regime.

Keywords: diet, glycogen replenishment, horses, respiratory exchange ratio, starch content

EFEITO DE DIETAS COM DIFERENTES NÍVEIS DE AMIDO SOBRE A CONCENTRAÇÃO DE GLICOGÊNIO DURANTE O TREINAMENTO E SUA REPOSIÇÃO APÓS EXERCÍCIO DE ALTA INTENSIDADE

RESUMO- O glicogênio muscular é uma importante fonte de energia e uma fator potencialmente limitante do desempenho equino. Devido ao grande número de estudos usando concentrados de baixo amido e alta fibra e gordura para o manejo de distúrbios metabólicos, o uso de tais concentrados se tornou prática popular. Entretanto, há pouca evidência sobre a eficiência de tais concentrados sobre a manutenção e a reposição das concentrações de glicogênio muscular. Para avaliar o efeito de três dietas com conteúdo diferente de amido sobre a manutenção do glicogênio muscular durante um período de treinamento e sobre a repleção das reservas de glicogênio após exercício de alta intensidade, seis cavalos Puro-Sangue Inglês previamente condicionados fisicamente foram usados em um delineamento Quadrado Latino 3x 3. Os cavalos receberam 1 kg/100 kg de PV/dia de um concentrado com conteúdo de amido alto (HS), moderado (MS) ou baixo (LS). A forragem foi fornecida a uma taxa de 1.25 kg/100 kg PV/dia em todos os tratamentos. Os cavalos foram treinados por 3 semanas e então foram submetidos a três dias de exercício intenso programado para depletar substancialmente as reservas de glicogênio, e foram subsequentemente observados durante 4 dias de recuperação. Biópsias musculares foram obtidas antes da depleção e 0, 24, 48 e 72 horas após a depleção. O primeiro dia de depleção consistiu em um teste incremental (IET) e sangue foi amostrado a cada etapa de velocidade para análise de glicose e lactato. Durante o IET os cavalos usaram uma máscara para verificação do consumo de Oxigênio (VO_2), da produção de Dióxido de Carbono (VCO_2) e do coeficiente respiratório (RER). Um teste submáximo de esforço foi realizado antes e depois da depleção das reservas de glicogênio para investigar alterações na utilização dos substratos energéticos em função da depleção. A glicose plasmática e o RER durante o IET não diferiram entre tratamentos. O lactato foi menor para o tratamento LS ($P<0.05$). O RER durante o teste submáximo foi

menor para o tratamento LS (P<0.05). Os tratamentos HS e MS apresentaram RER pós-depleção menores que os RER pré-depleção durante o teste submáximo (p<0.05). A repleção do glicogênio muscular foi menor para LS após 72 horas de recuperação (P<0.05). Concentrados de baixo teor de amido parecem ser ineficientes na reposição de glicogênio muscular após a realização de exercício de alta intensidade, apesar de serem capazes de sustentar as concentrações de glicogênio durante um regime de treinamento regular.

Keywords: amido, cavalos, coeficiente respiratório, dieta, reposição de glicogênio

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
ART	Aerobic Respiratory Test
AUC	Area under the curve
DEP	Depletion protocol
GI	Glycemic index
GS	Gastroscopy scoping
HR	Heart rate
HS	High starch diet
IET	Incremental exercise test
LS	Low starch diet
LW	Light work routine
MB	Muscle biopsy
MS	Moderate starch diet
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
NSC	Non-structural carbohydrate
OFF	Day off from work
RER	Respiratory exchange ratio
TE	Treadmill exercise
VCO2	Carbon dioxide consumption
VFA	Volatile fatty acid
VLa2	Speed at which lactate was 2 mmol/l
VLa4	Speed at which lactate was 4 mmol/l
VO2	Oxygen consumption
WE	Walker exercise

1. INTRODUCTION

Horses are phenomenal athletes, and much of their athletic performance relies on efficient utilization of energy substrates, the most important of which is glycogen stored in muscles. It has been constantly verified that normal glycogen replenishment in horses takes up to 72 hours. In contrast, humans replenish glycogen stores within 24 hours. It has been demonstrated that horses trained on a daily basis might work under conditions of constantly depleted glycogen reserves, and that most equestrian competitions cause a significant reduction in glycogen reserves. Traditionally, replenishment of glycogen reserves consists of feeding large quantities of starch-rich meals of grain or grain-mixes. This strategy, however, increases the probability that non-digested starch will reach the hindgut, which could lead to gastric and metabolic disturbances such as colic and laminitis. Therefore, finding feeds, supplements and strategies that maximize glycogen resynthesis and allow for faster, more efficient replenishment of glycogen stores is of high importance.

Several studies have looked at different strategies for either sparing or hastening the resynthesis of glycogen. The effects of adding fat to the athlete horse's diet have been investigated and produced controversial effects. Some researchers found increased glycogen levels after fat supplementation, while others found no effect, and yet others found a decrease in glycogen concentrations with the addition of fat. It is thought that adding fat causes a glycogen-sparing effect by allowing horses to utilize more fat as an energy source during aerobic activity. Similarly, the substitution of starch for fermentable fibers such as beet pulp is also believed to provide alternative substrate through free fatty acids, also sparing glycogen reserves, although very few studies have investigated the effect of fermentable fibers on glycogen concentrations.

Over the last few years, in light of all the published research on feeding strategies for horses with metabolic diseases such as recurrent exertional rhabdomyolysis, polysaccharide storage myopathy and insulin resistance, the use of "low-starch" concentrates has become popular amongst riders in an attempt to reduce the risks of gastrointestinal and metabolic diseases, and many top-competing horses are on this type of feed. However, there is still scarce scientific evidence about the effects of such feeds

on the maintenance of glycogen stores during training or their potential to replenish glycogen stores after a substantial depletion, like that promoted by a high-intensity competition effort.

In this study, we aimed to investigate the effect of three different diets with varying starch content (low starch – LS; medium starch – MS and high starch- HS) on the maintenance of glycogen reserves throughout a three-week training regime and on the rate of replenishment following a glycogen-depleting (approximately 35% of initial reserves) exercise protocol. Choice of energy substrate was also investigated by measuring respiratory exchange ratios both prior to and after muscle glycogen depletion occurred.

2. LITERATURE REVIEW

The horse's high athletic ability can be attributed to its high aerobic capacity and vast energy substrate reserves, most particularly muscle glycogen. Glycogen stores are considered as the most important fuel for exercise, because they are abundant and can be used by both aerobic and anaerobic metabolism.

Muscle glycogen replenishment in horses is traditionally achieved through consumption of large quantities of concentrate rich in starch. Researchers have tried to better understand the glycogen mobilization and replenishment mechanisms of horses, as well as to find alternatives to feeding large amounts of starch as a means to restore glycogen reserves.

Results on the effect of equine diet manipulation over glycogen replenishment are still controversial, and there is need of further research to assess the role of starch on glycogen replenishment.

The horse, as other mammals, obtains energy from conversion of chemical energy in feeds into mechanical energy for muscular contraction in the form of ATP. Because horses cannot feed while exercising, energy must be stored for later use. Energy substrate utilization pathways are determined through several factors. Among the principal are duration and intensity of exercise. Long duration activities at low speed, known as endurance activities, are sustained almost exclusively by aerobic metabolism energy generation. In such activities, of which Endurance riding is the best example, the preferred energy generation pathway is oxidative phosphorylation, mainly of lipids and carbohydrates. Short duration, maximum speed activities, on the other hand, predominantly use anaerobic glycolysis to generate energy during exercise (MARLIN and NANKERVIS, 2002).

Efficiency of energy production from glycogen is much greater in aerobic mode. When glycogen is metabolized aerobically, ATP production per glucose unit is 13 times greater than that obtained through anaerobic pathways (EATON, 1994).

According to Snow and Valberg (1994), another factor influencing energetic metabolism and substrate preference is the horse's physical conditioning status. Horses trained for endurance have augmented oxidative muscular capacity, both through greater volume and density of mitochondria and through higher proportion of Type IIA muscular fibers (of high oxidative capacity) in relation to Type IIX fibers (of low oxidative capacity).

Diet composition also affects energetic metabolism. Several authors (MEYERS et al., 1989; OLDHAM et al., 1990; ORME et al., 1997) verified that diets with high inclusion of lipids, associated with adequate adaptation, promote higher efficiency of lipid utilization as an energy substrate.

The importance of glycogen for exercise maintenance has been widely researched, both in humans and in other species, including horses. According to Harris (1997), the main energy reserves in the horse are adipose tissue, which contains approximately 35,000 a 40,000 grams of triacylglycerol; triacylglycerol present in the muscle, adding up to 3,000 to 4,000 grams; and glycogen, present in the liver (about 100 to 200 grams) and in muscle (with reserves reaching about 4,000 grams). These values have been estimated based on a 450 kg horse, and may vary according to age, breed, size and physical condition. Although not the most abundant, glycogen stores are the most important source of energy generation during moderate to intense exercise in horses. This happens because the triglycerides reserves in adipose tissue, the greatest potential energy reserve, cannot be mobilized anaerobically. Furthermore, although it produces more ATP per kilogram of substrate, speed at which lipids are converted into energy is not enough to sustain fast muscle contraction (HARRIS, 1999).

Exercise promotes significant reductions in glycogen reserves of horses, varying with intensity and duration of the effort. In a study conducted by Snow and Harris (1991), researchers observed that regular training workouts of racehorses promoted a reduction of approximately 19 to 25% in muscle glycogen stores, while total quantities used in an actual race can reach 33% of initial reserves. Horses that undergo submaximal exercise for long periods, such as endurance horses, can have 56% (SNOW; BAXTER; ROSE, 1981) to 70% (HODGSON et al., 1983) of total glycogen stores depleted. Several other authors

(BRÖJER et al., 2006; LINDHOLM; PIEHL, 1974; VALBERG, 1986) report that trotter horses potentially work daily with their glycogen reserves partially depleted.

Availability of glycogen reserves before the onset of exercise seems to have an effect over equine athletic performance, although results are still conflicting. Some studies established that a low glycogen concentration at the start of exercise diminishes aerobic capacity of horses (DAVIE et al., 1996), but not their anaerobic capacity (SYMONS; JACOBS, 1989). In contrast with these results, other researchers (LACOMBE et al., 1999, 2001; TOPLIFF; POTTER; DUTSON, 1983) found a direct correlation between initial glycogen content and anaerobic capacity.

Glycogen replenishment in horses obeys a complex regulatory system, which involves several enzymes and precursors, such as glycogen synthase, blood glucose and insulin. Muscle glycogen replenishment rate found in horses varies considerably. In humans, replenishment of glycogen reserves is two to three times faster, being completed in 24 hours (COSTILL et al., 1981).

Therefore, muscle glycogen replenishment is of indisputable importance for performance horses. This includes horses submitted to a daily training regime, such as trotters and racehorses who may not have enough time to fully replenish their reserves; horses undergoing exercise that require repeated and consecutive efforts, such as Three-day eventing (HODGSON et al., 1984); and horses submitted to long duration effort which substantially deplete glycogen reserves, such as Endurance horses, as reported by Snow, Baxter and Rose (1981) and Snow et al. (1982).

Athletic performance of horses depends highly on their ability to sustain exercise, which is intimately linked to energetic reserves and their utilization efficiency (DAVIE et al., 1999; HODGSON et al., 1983 e 1984; JOSE-CUNILLERAS; TAYLOR; HINCHCLIFF, 2004; LACOMBE; HINCHCLIFF; TAYLOR, 2003a; SNOW; BAXTER; ROSE, 1981; SNOW; HARRIS, 1991.).

Several studies have been done with the purpose of determining the best ways to replenish glycogen in horses, evaluating aspects related to energetic metabolism, feeding management and energy feed sources. Great part of these studies derives from experiments with humans and other mammals like rodents and dogs. However, equine

energetic metabolism bears a few important differences in comparison to that of humans and other mammals, for the most part making it impossible to apply the same performance-improving strategies proven for those species to horses. Glycogen replenishment rate is one of such differences. Several researchers (HYYPPÄ; RASANEN; POSO, 1997; LACOMBE et al., 2004; SNOW; HARRIS, 1991) proved that horses submitted to high-intensity exercise which substantially depleted reserves take 48 to 72 hours to return glycogen levels to their pre-exercise status. In humans, according to COSTILL (1981), muscle glycogen is completely replenished after 24 hours.

It appears quite evident that not all conclusions drawn about human exercise physiology apply to horses, and that there is a clear need for more specific research in the equine species.

Muscle glycogen is the main energy generating substrate during submaximal efforts of long duration and short duration, high-speed exercise, once glycogen can be utilized both by aerobic and anaerobic pathways (HYYPPÄ; RASANEN; POSO, 1997; SNOW; BAXTER; ROSE, 1981; SNOW; HARRIS 1991; LACOMBE et al., 1999, 2001). Various authors (HODGSON et al., 1985; SNOW; HARRIS, 1991; VALBERG et al., 1985) verified that horses submitted to continuous training efforts depend on an efficient replenishment of glycogen stores to maintain good performance.

Currently, the most used strategy to restore glycogen reserves is supply of meals rich in starch. However, according to Waller and Lindinger (2010), because horses evolved as hindgut fermenting herbivores, depending largely on fermentation of fibrous carbohydrates to supply energetic demand, they seem to have a limited capacity to digest hydrolysable carbohydrates (starch). Results of recent research demonstrate that the slow speed in glycogen resynthesis compared to humans after oral supply of hydrolysable carbohydrates may be related to the fact that horses present limiting differences in carbohydrate absorption from the gut in comparison to humans, aside from also having a lower capacity to utilize glucose in the skeletal muscle (GEOR et al. 2006; PRATT et al., 2007).

Therefore, there is great concern for better understanding of energy generation metabolism and muscle glycogen replenishment in horses, and for developing alternative

feeding strategies that provide more efficient and faster replenishment, without resorting to the potentially harmful strategy of feeding large quantities of hydrolysable carbohydrates.

Several authors have studied the effects of supplying meals rich in starch after exercise. Some authors (LACOMBE et al., 2004; SNOW et al., 1987; TOPLIFF; POTTER; DUTSON, 1983; TOPLIFF et al., 1985) verified the existence of an acceleration effect in post-exercise glycogen replenishment rate with a diet rich in hydrolysable carbohydrates, but this effect was quite modest if compared to results of similar diets in humans (JENTJENS et al., 2001). However, some of these studies did not use isocaloric diets (SNOW et al., 1987; TOPLIFF; POTTER; DUTSON, 1983; TOPLIFF et al., 1985;), and the positive effect may be related to the greater supply of energy provided by diets in the "high-starch" treatment. Lacombe et al. (2004) investigated the effect of diets with different levels of hydrolysable carbohydrates fed after exercise on the replenishment of glycogen. Horses received either a starch-rich diet composed of grains only, a mixed diet comprised of grain and hay or a diet with low starch content composed of hay only, immediately after a glycogen-depleting exercise protocol that reduced glycogen reserves by at least 60%. The starch-rich diet accelerated glycogenesis in comparison to the other diets and was the only one able to promote return of glycogen concentration to pre-exercise levels after 72 hours. Jose-Cunilleras et al. (2006) studied the effect of starch-rich meals (2.7 g of starch/kg BW) on post-exercise glycogen replenishment, and obtained a modest increase (from 8 mmol/kg to 12 mmol/kg) in replenishment rate with the starch-rich treatment. However, it is worth noting that according to Meyer et al. (1995) and Potter et al. (1992), the safe established limit for starch consumption in horses is 2 to 4 g of starch/kg BW/meal, and the amounts used in this study are commonly associated with gastrointestinal and metabolic disorders that can lead the horse to death. More recently, researchers have established that the safest starch consumption should remain at less than 1.1 g of starch/kg BW/meal (VERVUET et al., 2009). This recommendation enhances the risk of gastric disturbances of meals with more than 2 g of starch/kg BW/meal, frequently used in trials which try to hasten glycogen replenishment.

Therefore, there is still not enough evidence to support the hypothesis that supply of meals with high quantities of starch promote a positive effect on glycogen

replenishment after exercise which can compensate the risks of gastric disorders such as colic and ulcers and metabolic diseases such as laminitis, recurrent exertional rhabdomyolysis and insulin resistance (GARNER et al., 1975; HOFFMAN et al., 2003; MURRAY, 1994; TINKER et al., 1997).

Consistent with the hypothesis that ingestion of substantial amounts of starch could generate a positive effect on glycogen replenishment, some studies verified the efficiency of supplying intravenous glucose after exercise as a strategy to accelerate glycogen replenishment rates. In studies conducted by Davie et al. (1996), Geor et al. (2006) and Lacombe et al. (2001, 2003b), administration of intravenous glucose during the 12 hours following end of exercise promoted faster glycogen replenishment.

Snow et al. (1987) reported a rate of 12.5 mmol/kg dw/hour for the first 8 hours after infusion of glucose associated to a starch-rich meal. Hodgson et al. (1984) found rates of 5.6 mmol/kg/h and 7.8 mmol/kg/h for the first 20 to 24 hours that followed a 160 km endurance race and for the first immediate hours after a treadmill test, respectively. Replenishment rates of 19.8, 14.6 and 7.1 mmol/kg dw/h were reported respectively for the first 6, 12 and 24 hours following end of exercise by Davie et al. (1996), after administration of intravenous dextrose.

Fast fermentable fibers, popularly known as “superfibers”, are fibrous carbohydrates with high fermentation capacity that produce energy in quantities similar to the same amount of starch. In the carbohydrate fractioning proposed by Hoffman et al. (2001), superfibers are represented by fructanes, pectins, resins and mucilages. The most studied fonts of superfibers include beet pulp, soy hulls and citrus pulp. Inclusion of superfibers in the diets and concentrates of horses has become popular because it allows for substitution of part of the starch in the diet, without great loss of energy, thus avoiding risks associated with high amounts of grain (and therefore starch).. Utilization of superfibers has found great success in the feeding management of horses suffering from metabolic syndromes related to glucose and insulin, such as insulin resistance, recurrent exertional rhabdomyolysis (MACLEAY et al., 2000) and polysaccharide storage myopathy (GEOR, 2005).

Some studies (LINDBERG; PALMGREN KARLSSON, 2001; WILLIAMS et al., 2001) investigated the effect of feeding superfibers on the energy metabolism and glycogen. It was observed that substitution of oats for *in natura* beet pulp promoted a lower glycemic and insulinemic response. In a later study, Palmgren Karlsson et al. (2002) found that substitution of part of the starch in the diet for beet pulp associated to molasses produced a lower reduction of glycogen reserves after an exercise test and lower lactate post-exercise. This effect is possibly related to the greater production of the free fatty acids acetate, propionate and butyrate, products of the fermentative digestion of fibers. The increased free fatty acids in this study may have acted as an alternative substrate for aerobic energy, thus providing a sparing effect on glycogen, similar to that observed when fat was added to the diet. Acetate is a major substrate for lipid synthesis, and can be immediately used in peripheral tissues. Previous research by Doreau et al. (1992) has demonstrated that a diet rich in forage and low in grain will alter production of volatile fatty acids (VFA) in favor of acetate.

Fat supplementation has been studied as an alternative to providing energy substrate without resorting to large starch quantities. Adaptation to a fat-supplemented diet has been shown to increase the horse's capacity to uptake non-esterified fatty acids (NEFA) from circulating triacylglycerol, as demonstrated by increased lipoprotein lipase activity (GEELEN et al., 2000; ORME et al., 1997). Horses adapted to dietary fat supplementation also showed lower respiratory exchange ratios during submaximal exercise, indicating higher capacity to utilize NEFA (DUNNET; MARLIN; HARRIS, 2002; PAGAN et al., 2002). Inclusion of fat in the diet promoted varied results in several other studies. According to Waller and Lindinger (2010), these controversial results probably occurred due to differences in amounts and type of lipid used, duration of the adaptation period, conditioning and low number of horses in studies. Some researchers reported a positive effect on the concentration and mobilization of glycogen reserves (MARQUEZE; KESSLER; BERNARDI, 2001; SCOTT et al., 1992; TREIBER et al., 2008), while others (EATON; HODGSON; EVANS, 1995; ESSÉN-GUSTAVSSON et al. 1991; GREIWE et al. 1989; HODGSON et al. 1986) did not find the same effect. Positive effects were mainly attributed to greater utilization of lipids during aerobic stages of exercise, thus preserving the glycogen reserves for the anaerobic stages in which it would be the most important

energy substrate, and promoting this way a glycogen-sparing effect. Excessive lipid levels in the diet, however, appear to compromise glycogen reserves. In a study that provided more than 15% of total diet in the form of lipids, Pagan et al. (1987) reported that glycogen reserves were reduced by almost 15%, It is recommended, therefore, that the percentage of lipids in the total diet does not exceed 10 to 12%.

Although some studies were successful in producing faster glycogen replenishment rates, especially in the first hours following exercise, such results are still not considered conclusive. Furthermore, the majority of the strategies researched has little practical relevance.

There is a clear need of research about ingredients and feeding protocols and strategies that can be easily applied to the sport horse industry.

3. OBJECTIVES

a. General

To evaluate the effect of three diets with different starch content on muscle glycogen content during training and on replenishment after strenuous exercise in horses.

b. Specific

i. To evaluate the efficiency of diets with different content of starch on the maintenance of glycogen reserves over a period of training.

ii. To assess the effect of different starch content in the diet on the replenishment on muscle glycogen reserves after substantial depletion.

iii. To investigate the effect of diets with different starch content on the utilization of energy substrates during exercise, before and after depletion.

4. MATERIAL AND METHODS

Experimental design

The effects of three diets with varying levels of starch on the maintenance and replenishment of glycogen reserves were evaluated in a 3 x 3 replicated Latin Square design. Six horses were subjected to a training period of 3 weeks. They then underwent a 3-day glycogen depleting exercise protocol, which consisted of an incremental exercise test (IET) on day one and high-intensity interval workouts on the two subsequent days. A graphic representation of the trial timeline is shown in Figure 1. The 3-week training regime consisted of alternated treadmill exercise (TE) and walker exercise (WE), with one day off per week out to pasture (OFF). On the third week, horses performed the last of their regular walker routine on the treadmill in a submaximal test (pre-ART) wearing a mask for gas exchange measurements. On the fourth week, horses were submitted to a glycogen-depletion protocol. Depletion protocol consisted of an incremental exercise test (IET) on day one, followed by two days of intense interval exercise on the treadmill (DEP). Blood was collected from the jugular vein during the IETs through a catheter for determination of glucose and lactate levels at each of the speed steps in the test. Horses wore a loose-fitting mask during the IETs for measurement of VO2 and VCO2 and determination of respiratory exchange ratio (RER) through the use of an open circuit indirect calorimeter. Muscle biopsies (MB) were obtained before depletion and 0, 24, 48 and 72 hours post-depletion. A second submaximal RER test (post-ART) was performed immediately after the third day of depletion to verify changes in substrate utilization with depleted glycogen stores. During the washout, horses were kept on a light-work (LW) training regime which excluded the 10 m/s gallop in the treadmill workout; they also underwent gastroscopy for assessment of ulcerations.

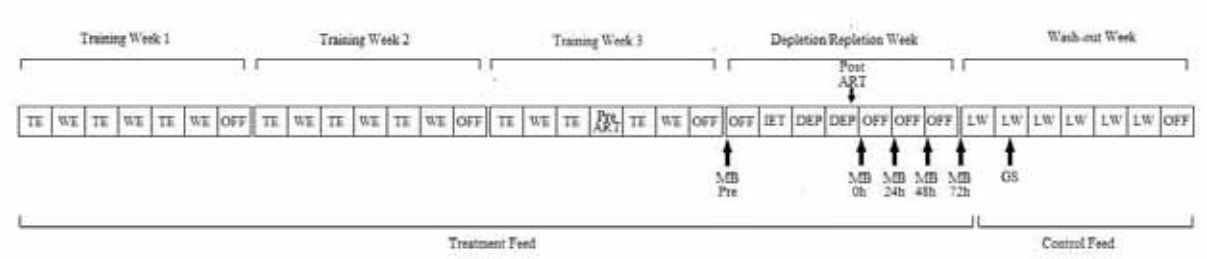

Figure 1. Graphic representation of trial timeline. Horses were trained for 3 weeks, alternating exercise on the treadmill (TE) and walker (WE) and had one day off per week (OFF). On the third week, horses performed an aerobic RER test (Pre ART) on treadmill. On the fourth week, horses were submitted to a 3-day glycogen-depletion protocol, day one being an incremental exercise test (IET), and days two and three being intense interval exercise on the treadmill (DEP). Muscle biopsies (MB) were obtained before and 0, 24, 48 and 72 hours post-depletion. On the afternoon of depletion day three horses went back on the treadmill for a second ART to assess changes in substrate utilization after depletion (Post ART). During the washout, horses were kept on a light-work training schedule (LW) and were checked for gastric ulcerations (GS). This trial was repeated over three periods during the study.

Horses

Six adult, fit Thoroughbred horses weighing 555.2±29.9 kg (Mean±SD) and aged 8.2±2 years old (Mean±SD), were used in a replicated 3 x 3 Latin square design. Horses were pre-conditioned on a similar training schedule to the one used during the washouts for two months prior to the commencement of the study and were fit and used to work on the treadmill and automatic walker upon the start of the first period of the trial.

Glycemic Index of Concentrates

The glycemic response of the three experimental concentrates was determined in a separate study, in which 4 horses were arranged in a 4x4 Latin Square design and received a 1 kg meal of one of the three feeds or oats and blood samples were collected before and at 0, 30, 60, 90, 120, 150, 180, 210 and 240 minutes after the meal for determination on glucose values. Oats was used as the indexing feed and glycemic indexes were expressed as $AUC_{feed}/AUC_{oats.}$

Concentrate and Diet Composition

Three concentrates with varying levels of starch were tested in this study. A low starch, high fat and fiber mix (LS), an intermediate starch and fat content mix comprised greatly of dehydrated corn germ (MS) and a traditional sweet feed, high-starch mix (HS). Concentrates were fed at a rate of 1 kg per kg BW per day. The forage portion of the diet was composed of Northern Timothy hay, fed at 1.25 kg per kg BW per day. Concentrates and forage were submitted to Dairy One (Dairy One Inc., Ithaca, NY) for nutrient analysis (Table 1). Nutrient composition was determined by near infrared reflectance spectroscopy. Hemicellulose and NSC were calculated from determined values, as was Digestible Energy. Total diet nutrient composition is presented in Table 2 and concentrate centesimal composition is given on Table 3. Diets were isocaloric and isonitrogenous. To ensure proper electrolyte reposition, horses were fed an electrolyte supplement daily (Endura-Max, Kentucky Equine Research, Inc., Versailles, KY). Horses also received a mineral-vitamin supplement (Micromax, Kentucky Equine Research, Lexington, KY) at 150 grams per horse per day to provide adequate micronutrients, since concentrates did not contain a mineral-vitamin premix. Diets were calculated from NRC (NATIONAL RESEARCH COUNCIL, Nutrient requirements of horses. 6.ed, 2007) energy demand values for very heavy exercise. Forage:concentrate ratio was 56:44 in total diets.

Table 1. Nutrient analysis of Diet Concentrates (LS=Low-starch; MS=Medium Starch; HS=High starch) and Hay (Timothy Grass), on a dry matter basis.

Nutrient		LS	MS	HS	HAY
Moisture	%	10.8	9.6	11.6	10.0
Dry Matter	%	89.2	90.4	88.4	90.0
Crude Protein	%	12.0	11.8	9.4	10.2
Digestible Energy[1]	Mcal/kg	3.3	3.6	3.6	2.7
ADF	%	41.5	15.7	7.3	39.7
NDF	%	59.0	24.8	14.1	55.5
Hemicellulose[2]	%	17.5	9.1	6.8	15.8
Starch	%	6.4	39.1	56.9	0.7
Sugars	%	5.9	7.4	8.3	11.3
NSC[3]	%	12.3	46.5	65.2	12.0
Crude Fat	%	14.3	7.8	4.8	3.0
Ash	%	5.9	4.1	3.5	7.5

[1], [2], [3] Calculated values.

ADF=Acid Detergent Fiber; NDF=Neutral Detergent Fiber; NSC=Non-structural carbohydrates.

Table 2. Nutrient composition of total (concentrate+forage) diets (LS=Low-starch; MS=Medium Starch; HS=High starch) on a dry matter basis.

Nutrient		LS	MS	HS
Moisture	%	10.4	9.8	10.7
Dry Matter	%	89.6	90.2	89.3
Crude Protein	%	11.0	10.9	9.8
Digestible Energy[1]	Mcal/kg	3.0	3.1	3.1
ADF	%	40.5	29.0	25.3
NDF	%	57.1	41.9	37.1
Hemicellulose[2]	%	16.6	12.8	11.8
Starch	%	3.2	17.8	25.7
Sugars	%	8.9	9.6	10.0
NSC[3]	%	12.1	27.3	35.6
Crude Fat	%	8.0	5.1	3.8
Ash	%	6.8	6.0	5.7

[1, 2, 3] Calculated values.

ADF=Acid Detergent Fiber; NDF=Neutral Detergent Fiber; NSC=Non-structural carbohydrates.

Table 3. Centesimal composition of tested concentrates (LS=Low-starch; MS=Medium Starch; HS=High starch).

Ingredient	LS	MS	HS
Cracked corn			45
Oats			45
Molasses	2.5		10
Fiber pellet	67.5		
Wheat middlings	*40*		
Soy hulls	*47.5*		
Binder (lignosulfonate)	*2.5*		
Vegetable oil	10		
Beet pulp	20		
Dehydrated corn germ		85	
Dehydrated alfafa meal		15	

Feeding schedule

Horses were fed concentrate twice daily, at 7am and again at 4 pm, at a rate of 1% kg BW/day. They also received 1 kg of grass hay (Northern Timothy) at 7am, 2 kg at 4 pm and the remainder of their calculated 1.25% kg BW daily amount at 10pm. On pre-ART days, horses were fed their usual 1 kg hay plus morning concentrate meal one hour earlier than usual (at 6am) to allow enough time between feeding and the pre-ART, which started three hours later. On the first day of the depletion protocol, when horses performed the IET, concentrate was withheld altogether before the tests, and horses only received 1 kg of hay prior to the test. Concentrate was offered to the horses as soon as they had been cooled off after finishing both the IET test. On the last day of the depletion protocol, horses received 1 kg of hay prior to their interval treadmill workout. They then

returned to the treadmill for the post-depletion ART test. Concentrate was withheld until they had finished the post-ART test and had their muscle sampled. During the 72-hour recovery period horses were fed their experimentally assigned concentrate and remained at rest, being turned out daily during mornings and afternoons.

Training protocol

During the initial three weeks of each experimental period, horses were submitted to a training regime. The weekly workout schedule consisted of three days per week of treadmill interval workouts alternated with three days of walker exercise (Table 4).

Table 4. Exercise protocol during training period.

Exercise[1]	Description of exercise
High-speed treadmill[2] (every other day, 3 times/week)	5 min warm-up at the walk (1.7m/s), 5 min at a trot (4m/s), 3 min at a canter (8m/s), 5 min at a trot (4 m/s), 2 min at a gallop (10m/s), 5 min at a trot (4m/s), 3 min at a canter (8m/s), 5 min at a trot (4m/s) and 5 cool-down at a walk (1.7 m/s).
Automatic walker (every other day, 3 times/week)	5 min at the walk (1.7m/s), 10 min at the trot (4m/s), reversal of direction, 10 min at the trot (4m/s) and 5 min at the walk (1.7m/s).

[1]All work during the training period was done with no incline on the treadmill. [2]During the first week of each period and during washouts, treadmill protocol excluded one step at the trot (4m/s) and the gallop (10m/s).

Glycogen-depleting protocol

On the fourth week of each experimental period, horses underwent a three-day, glycogen-depleting protocol. On day one, horses performed an incremental exercise test (IET) on an inclined treadmill (3° incline), consisting of a five-minute warm-up at the walk (1.7 m/s), followed by two minutes at 4, 6 and 8 m/s and 1 minute at 9, 10, 11, 12 and 13 m/s. At the end of the last galloping step, the treadmill was immediately brought to 0°

incline and horses were cooled off at the walk for five minutes. Horses then stood on the treadmill for a further five minutes so that a post-exercise blood sample was collected. On days two and three of the depletion protocol, horses were brought to the treadmill for an interval-training workout (Figure 2), consisting of 5 minutes at the walk (1.7 m/s) followed by 4 series of 5 minutes of trot (4 m/s) alternated with 2 minutes of gallop (10 m/s), with a cooling-off period of 5 minutes at the trot (4 m/s) and 5 minutes at the walk (1.7 m/s) (Table 5). Horses were divided in two groups and groups were staggered for better management of the trial.

Table 5. Three-day glycogen depletion protocol.

Day	Description of exercise
1* Incremental Exercise Test (IET)	5 minute warm up at the walk (1.7m/s), 2 minutes at each step of 4, 6 and 8 m/s, 1 minute at each step of 9, 10, 11, 12 and 13 m/s, 5 minutes cool down at the walk (1.7m/s) and 5 minutes rest standing on the treadmill
2* Intense Interval Exercise	5 minute warm up at the walk (1.7m/s), 4 series of 5 minutes at the trot (4m/s) and 2 minutes of gallop (10m/s), followed by a 5 minute cool down at the trot (4m/s) and 5 minutes at the walk (1.7m/s).
3* Intense Interval Exercise	5 minute warm up at the walk (1.7m/s), 4 series of 5 minutes at the trot (4m/s) and 2 minutes of gallop (10m/s), followed by a 5 minute cool down at the trot (4m/s) and 5 minutes at the walk (1.7m/s).

*All work on the treadmill during the depletion protocol was done on a 3° incline.

Figure 2. Picture showing exercise on the treadmill on the second day of the depletion protocol.

Body Weights

Horse's body weights were measured weekly throughout the study by use of a portable digital scale (Equimetrics Inc., Redfield, AR, USA).

Blood parameters

On the day of the IET, 14 gauge 13 cm catheters (MILA International Inc., Erlanger, Kentucky) were inserted intravenously in the jugular on the left side of all horses undergoing the IET prior to the test. Blood samples were collected before the start of the IET, at the end (final 15 seconds) of each speed increment, and five minutes after the end of the IET with the horse standing on the treadmill. Samples were centrifuged and plasma

was stored in plastic cryogenic storage tubes at -20°C until analysis for determination of lactate and glucose was performed. Plasma glucose and lactate were determined electrochemically using a Lactate and Glucose analyzer (YSI 2300 Stat Plus, Yellow Springs Instruments).

Glycogen concentration/Muscle biopsies

Biopsy specimens were obtained from the middle gluteal muscle within a 1-inch square of a standardized site 17 cm along a line running from the most dorsal part of tuber coxae to the head of the tail alternating limbs for each subsequent biopsy. A 6 mm diameter Bergstrom biopsy needle was used. A subcutaneous local anesthetic was injected, an incision made in the skin and the needle inserted to a depth of 60 mm. Biopsy specimens were immediately frozen in liquid nitrogen and stored at -80 degrees C until biochemical analysis was performed. Horses were sedated using 5 mg Detomidine per horse (Dormosedan, Pfizer Animal Health, New York, New York) intravenously and sampling area was anesthetized using Mepivacaine Hydrochloride 2%, 2-3 ml per horse, subcutaneously (Carbocaine-V, Pfizer Animal Health, New York, New York). Muscle samples (Figure 3) were obtained one day prior to commencement of the depletion protocol and then immediately after completion of the third day of depleting exercise and at 24, 48 and 72 hours thereafter. For assessment of glycogen content, frozen muscle specimens were weighed and portions (1-2 mg) of muscle tissue were boiled for 2 hours in 1 M HCl (LOWRY; PASSONNEAU, 1972). Glycogen was assayed fluorometrically in muscle biopsy specimens as glucose residues.

Figure 3. Muscle sampling. Samples were collected and frozen in liquid Nitrogen immediately after sampling. Samples were then stored at -80°C for later analysis of glycogen content.

VO_2 and VCO_2

Oxygen consumption (VO_2) and carbon dioxide production were measured with an open-circuit indirect calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH), both during the IETs and the pre- and post-ARTs. Flow through the system was ~1,500 l/min with the horse stationary and at the warm-up walk and 10,000 l/min during higher speeds. The oxygen and carbon dioxide sensors of the open-circuit calorimeter (electrochemical cell and single beam nondispersive infrared sensor, respectively) were calibrated against gases of known composition within 10 min of the start of each exercise test, during which the horse stood quietly on the treadmill. The overall accuracy of the system was verified daily by nitrogen dilution. Discrepancy between stimulated VO_2 produced by nitrogen dilution and the values measured by the system was 63% at nitrogen flow rates equivalent to a VO_2 of 54 l/min (~140 ml/min/kg for a 385-kg horse).

Respiratory Exchange Ratio (RER)

Horses were brought to the treadmill for a respiratory exchange ratio (RER) test on two occasions. First, on the third week of each period, horses performed the last of their usual walker exercise (WE) routine (5 minutes at the walk, 20 minutes at the trot, 5 minutes at the walk) that week on the treadmill instead, wearing a loose-fitted mask for measuring VO_2 and VCO_2. This test was then repeated after completion of the third and last day of the depletion protocol, in such a way that the first horse to complete the depletion protocol would return to his stall, be fed a hay meal and then return to the treadmill for the post-depletion RER at least 4 hours after feeding. RER was calculated as VCO_2/VO_2.

Heart rates

Heart rates were measured during the IET. An equine heart monitor (Polar Equine RS800 G3) was used for all heart rate measurements.

Statistical analysis

Statistical analysis was performed by One-way repeated measures ANOVA and individual differences were assessed by use of Tuckey's test.

5. RESULTS

During the first period, one horse suffered a rhabdomyolysis episode and had to be excused from the trial, all of its data being subsequently removed. All data for this study represent the five remaining horses.

Glycemic Index of tested feeds

A separate trial using four horses was done to evaluate the glycemic response of the tested concentrates. Oats was used as the indexing feed (Glycemic index=100). There was no significant difference ($p>0.05$) in glycemic index between HS, MS and oats. However, LS had a significantly lower ($p<0.05$) glycemic index than that of the two first feeds and of oats. Glucose values for the three concentrates used in this study, in addition to that of oats, used as the indexing feed, are shown in Figure 4. Glycemic index of feeds, calculated as area under the curve (AUC) of feed divided by AUC of oats, are shown in Table 6.

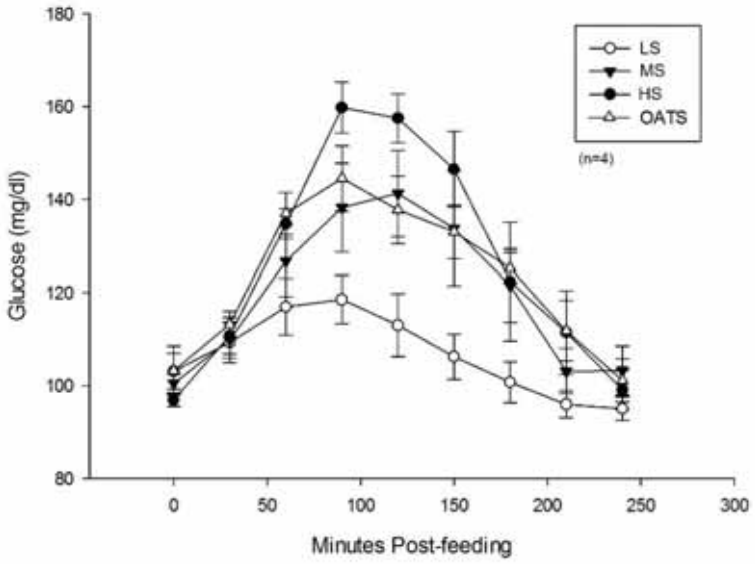

Figure 4. Glucose during Glycemic Index trial. Data shows glucose values at 0, 30, 60, 90,120, 150, 180, 210 and 240 minutes after feeding of 1 kg of each feed.

Table 6. Glycemic Index (GI) of trial concentrates (Mean± SD). GI was calculated as area under the curve (AUC) of tested feed divided by AUC of oats (indexing feed). (LS=low starch; MS=medium starch; HS=high starch).

	Feeds			
	LS	MS	HS	Oats
Glycemic Index (AUCconc/AUCoats)	31.75±12.66*	92.25±47.54	134±27.99	100±28.25

*Indicates difference from MS, HS and Oats ($p<0.05$)

Body Weights

Horses maintained body weights (Figure 5) throughout the study without significant changes ($p>0.05$).

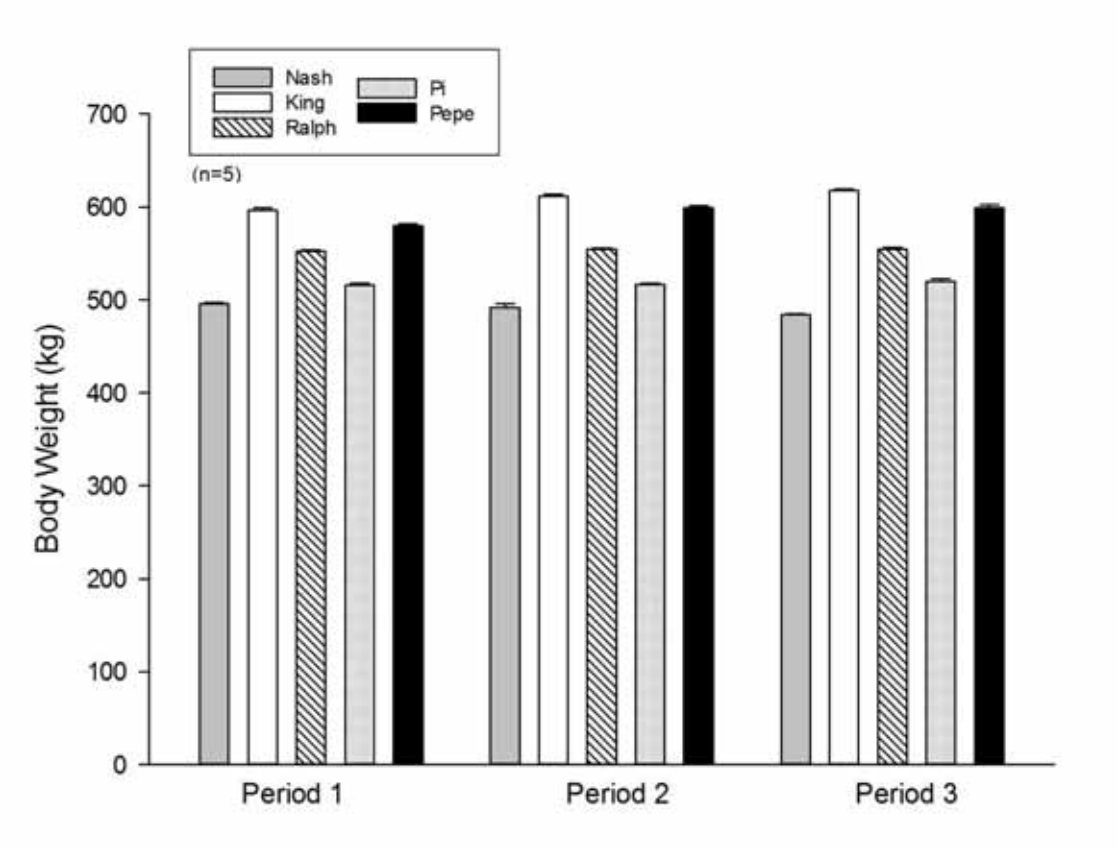

Figure 5. Body weights (Means±SE) of the five horses (Nash, King, Ralph, Pi and Pepe) used in the study throughout the three trial periods.

Blood parameters/Biochemical Analysis

Plasma Glucose and lactate levels were measured on the first day of the depletion protocol, which consisted of an incremental exercise test (IET). Lactates during the IET are shown in Figure 6. Lactate means were lower in the LS treatment ($p<0.05$) (Figure 7). Glucose was unaffected by treatment (Figure 8). Lactate and glucose values during the IET are shown in Table 7.

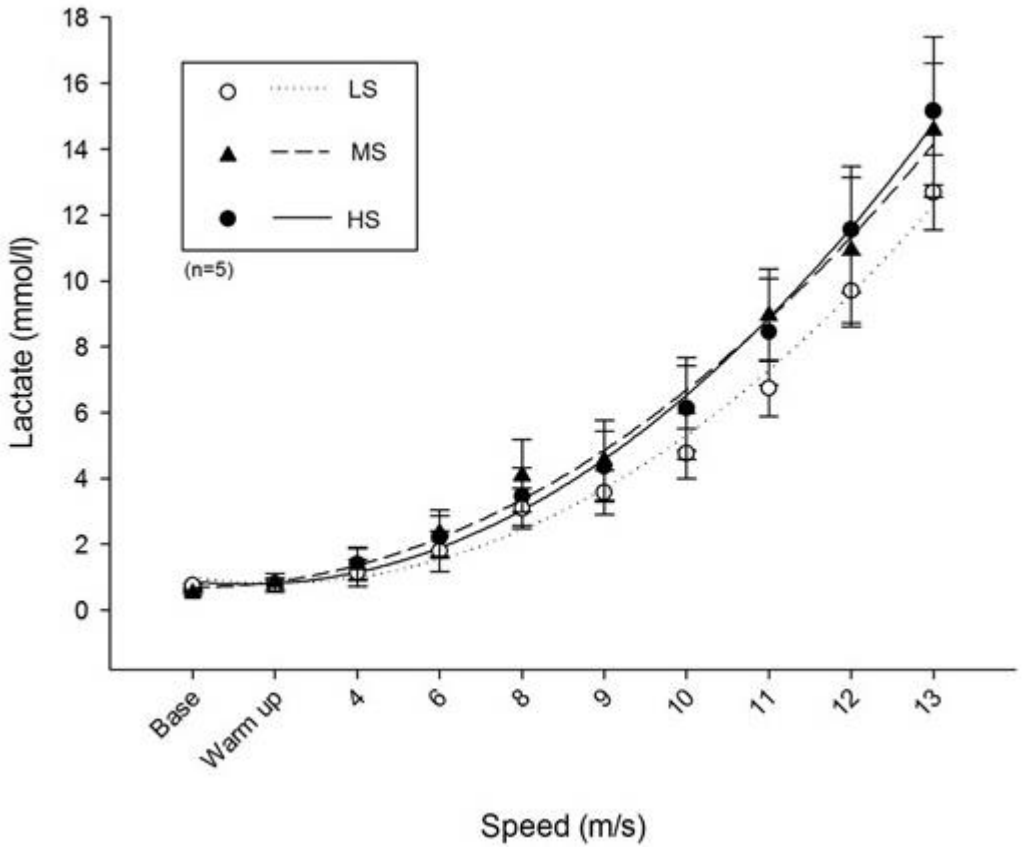

Figure 6. Lactates during the IET (Incremental Exercise Test) test for LS (low-starch), MS (medium starch) and HS (high-starch) treatments.

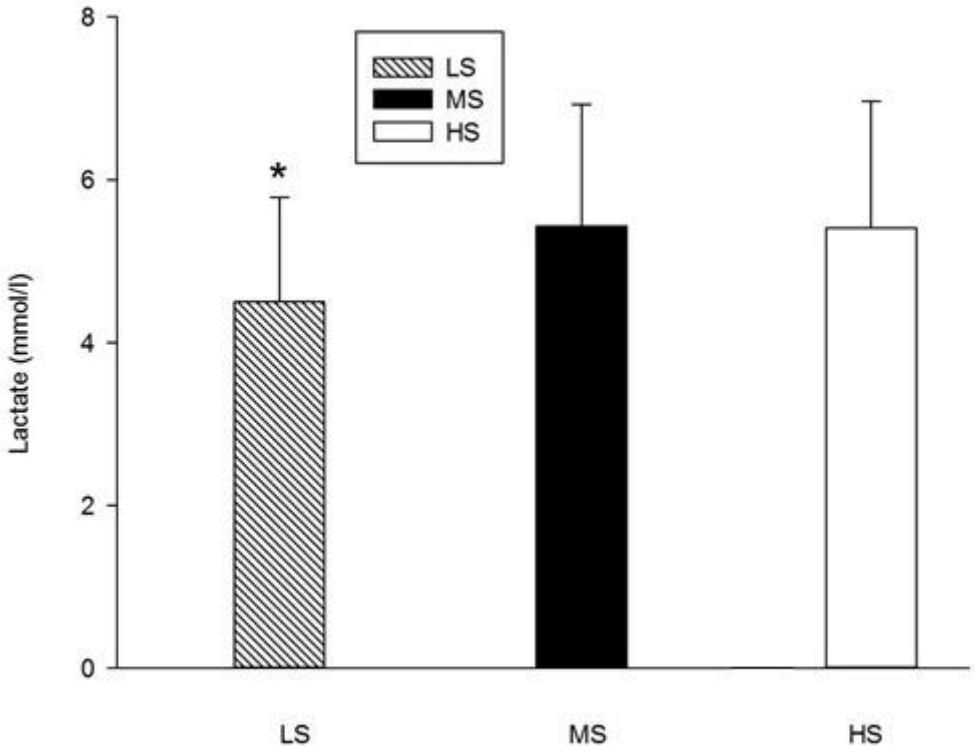

Figure 7. Lactate means during IET. (*) Indicates difference from HS and MS ($p<0.05$). (n=5)

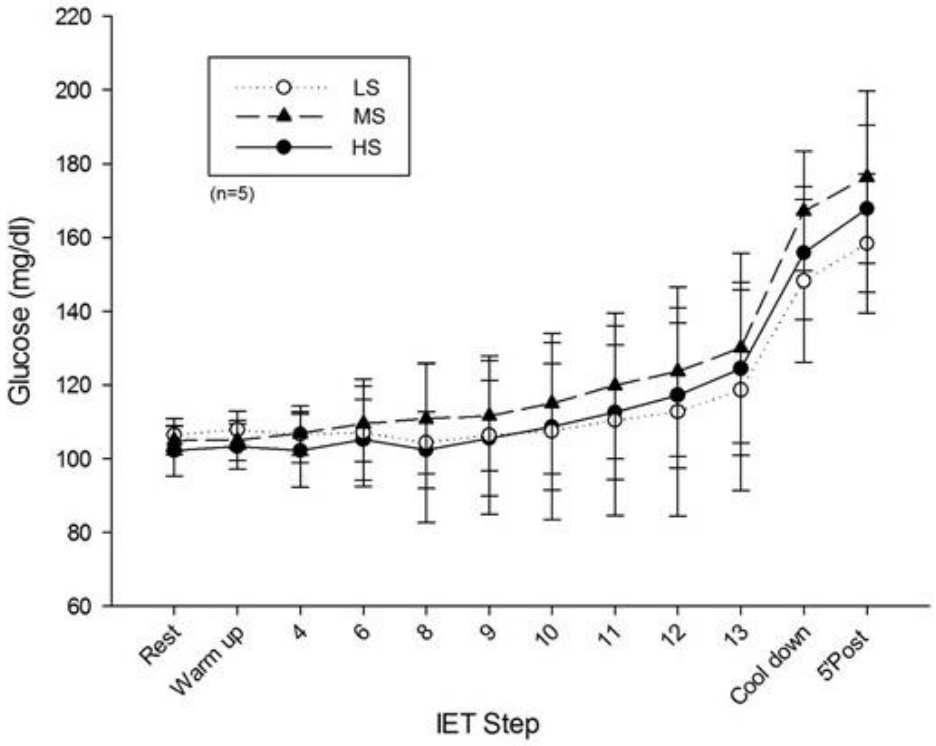

Figure 8. Glucose during the IET test. Values are Mean±SE for LS (low-starch), MS (medium starch) and HS (high-starch) treatments.

Lactate thresholds

Speed at which lactate was 2 and 4 mmol was determined by exponential regression from lactate values during the IETs. There were no differences ($p>0.05$) among treatments and results are presented in Figure 9.

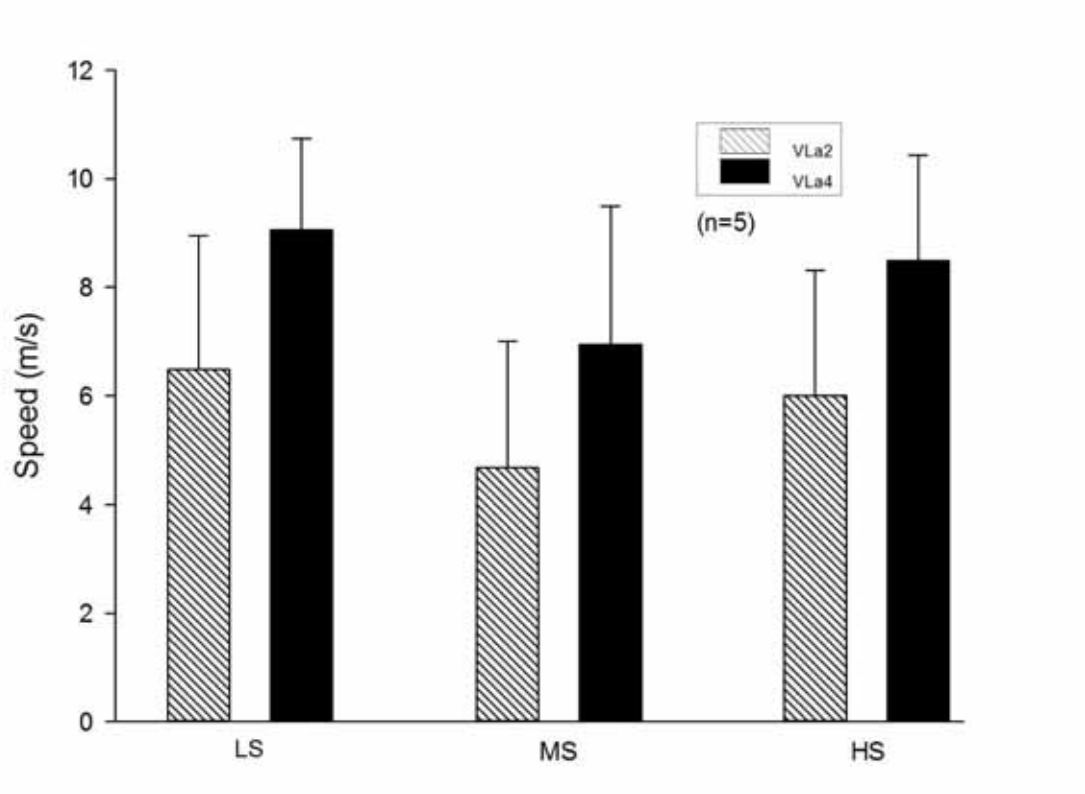

Figure 9. VLa2 and VLa4 for the low starch (LS), medium starch (MS) and high starch (HS) treatments.

Table 7. Blood parameters (glucose and lactate) during the Incremental Exercise Test (IET). Values are means±SD for LS (Low-starch), MS (Medium Starch)and HS (High-starch) treatments; (n=5).

		IET Step*											
Variable	**Treatment**	**Baseline**	**Walk 1**	**4**	**6**	**8**	**9**	**10**	**11**	**12**	**13**	**Walk 2**	**5'Post**
Lactate (mmol/l)	LS	0.74 ± 0.11	0.78 ± 0.34	1.13 ± 0.92	1.79 ± 1.37	3.09 ± 1.38	3.57 ± 1.49	4.76 ± 1.70	6.73 ± 1.90	9.7 ± 2.44	12.69 ± 2.54	13.87 ± 4.28	11.78 ± 2.57
	MS	0.52 ± 0.10	0.83 ± 0.63	1.39 ± 1.04	2.34 ± 1.55	4.08 ± 2.46	4.55 ± 2.70	6.12 ± 3.45	8.95 ± 3.14	11.53 ± 4.95	14.57 ± 4.54	16.26 ± 6.04	14.76 ± 7.18
	HS	0.59 ± 0.10	0.76 ± 0.46	1.40 ± 1.13	2.22 ± 1.43	3.45 ± 1.96	4.36 ± 2.40	6.13 ± 2.87	8.45 ± 3.60	11.56 ± 4.28	15.16 ± 5.04	16.54 ± 5.32	15.06 ± 6.05
Glucose (mg/dl)	LS	106.40 ± 4.39	107.80 ± 5.07	106.60 ± 7.70	107.02 ± 14.51	104.32 ± 21.64	106.36 ± 21.45	107.44 ± 24.04	110.38 ± 25.74	112.66 ± 28.26	118.6 ± 27.22	148.2 ± 22.17	158.4 ± 18.88
	MS	104.9 ± 3.88	104.96 ± 5.40	106.8 ± 5.89	109.48 ± 10.21	110.8 ± 14.92	111.6 ± 14.91	115,00 ± 19.09	119.8 ± 19.77	123.6 ± 23.01	130.0 ± 25.74	167.2 ± 16.24	176.4 ± 23.41
	HS	102.14 ± 6.79	103.34 ± 6.15	102.16 ± 9.92	105.16 ± 10.94	102.34 ± 10.42	105.56 ± 15.62	108.62 ± 17.13	112.6 ± 18.22	117.2 ± 19.65	124.4 ± 23.42	155.8 ± 17.95	167.8 ± 22.62

*Baseline: parameter at rest, before the test; Walk 1: Warm-up walk; Walk 2: Cool down walk; 4, 6, 8, 9, 10, 11, 12, 13: Speed at each incremental step, in m/s.

VO_2 and VCO_2 during IET test

There were no differences among treatments for VO_2 and VCO_2 during the IET test. Results for each parameter are represented in Figures 10 and 11, respectively. Mean values are shown in Table 8.

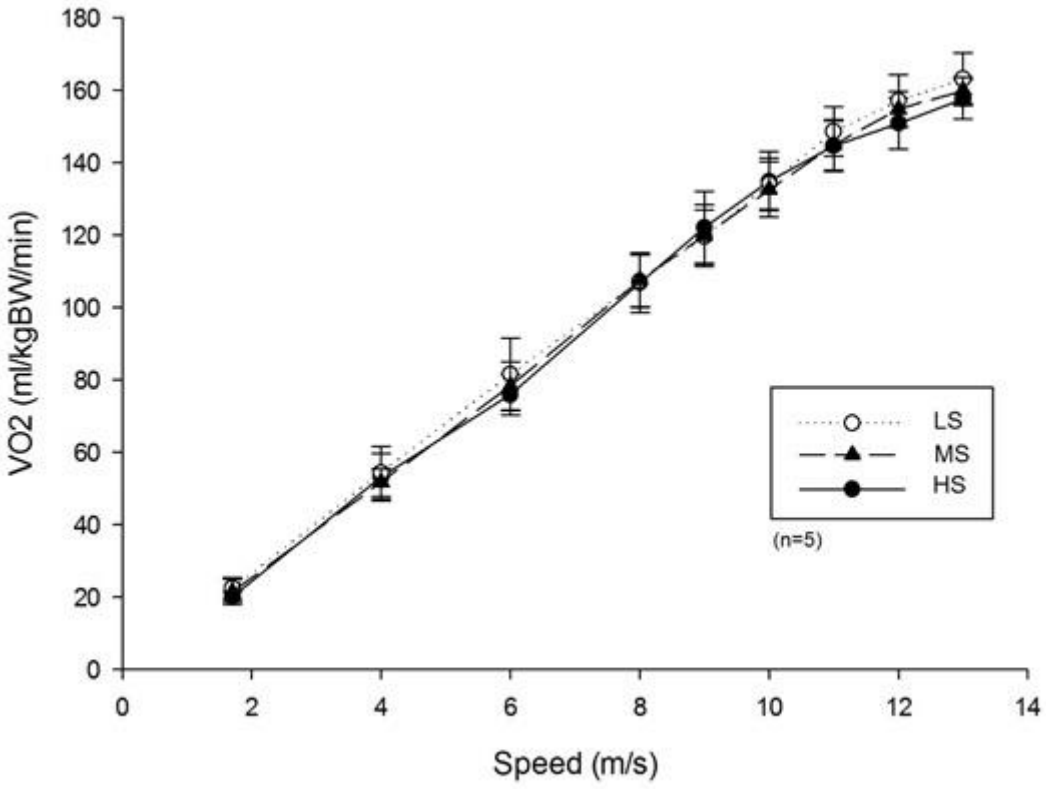

Figure 10. Oxygen consumption at the various speeds in the IET (VO_2) for the low starch (LS), medium starch (MS) and high starch (HS) treatments. Values are Means±SE.

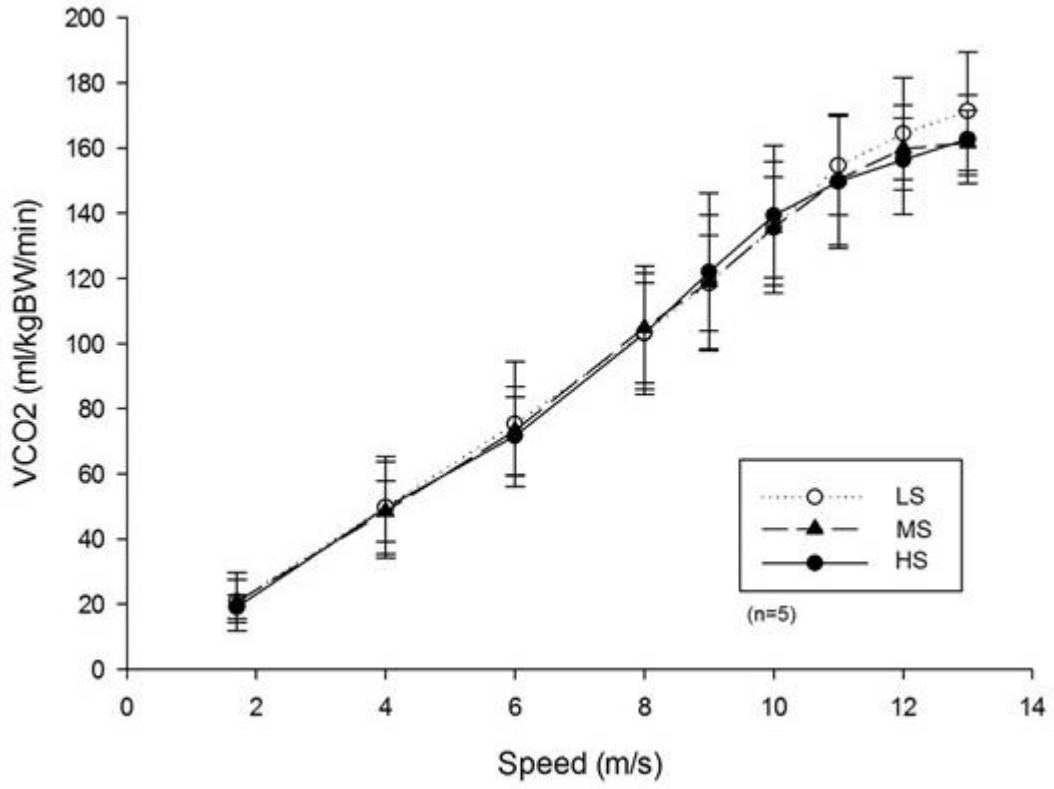

Figure 11. Production of CO_2 (VCO_2) at various the various speeds in the IET test for the low starch (LS), medium starch (MS) and high starch (HS) treatments.

Table 8. Cardio-respiratory parameters during the Incremental Exercise Test (IET). Values are Mean±SD for the low starch (LS), medium starch (MS) and high starch (HS) treatments. (n=5)

		Speed								
Variable	**Treatment**	**1.7**	**4**	**6**	**8**	**9**	**10**	**11**	**12**	**13**
VO2	LS	22.36 ± 6.88	54.40 ± 16.24	81.61 ± 22.22	107.34 ± 16.33	119.51 ± 16.61	134.16 ± 15.75	148.62 ± 15.16	157.09 ± 16.18	163.13 ± 16.16
	MS	22.01 ± 8.98	51.96 ± 9.78	79.73 ± 17.06	109.50 ± 18.53	120.03 ± 21.85	132.01 ± 19.65	144.28 ± 18.09	152.90 ± 11.66	158.94 ± 8.99
	HS	20.07 ± 3.16	53.11 ± 14.75	75.86 ± 12.46	106.77 ± 18.28	122.10 ± 22.17	134.93 ± 18.06	144.63 ± 15.57	150.90 ± 15.78	157.50 ± 12.07
VCO2	LS	20.94 ± 6.56	49.75 ± 15.48	75.25 ± 19.15	103.35 ± 15.41	118.56 ± 14.70	135.61 ± 15.44	154.57 ± 15.15	164.39 ± 17.14	171.28 ± 18.20
	MS	19.17 ± 10.43	42.50 ± 16.26	64.13 ± 24.79	91.04 ± 35.25	103.33 ± 39.00	116.97 ± 42.46	130.10 ± 46.36	136.63 ± 45.25	139.12 ± 45.16
	HS	19.23 ± 3.64	49.58 ± 14.10	71.57 ± 12.08	103.06 ± 18.63	122.01 ± 24.12	139.26 ± 21.45	149.69 ± 20.32	156.45 ± 16.72	162.74 ± 13.49
RER	LS	0.92 ± 0.20	0.91 ± 0.11	0.92 ± 0.12	0.98 ± 0.14	0.99 ± 0.07	1.01 ± 0.07	1.01 ± 0.08	1.02 ± 0.09	1.00 ± 0.08
	MS	0.96 ± 0.05	0.93 ± 0.04	0.94 ± 0.03	0.98 ± 0.03	1.00 ± 0.02	1.02 ± 0.02	1.05 ± 0.03	1.06 ± 0.02	1.03 ± 0.03
	HS	1.03 ± 0.21	0.94 ± 0.13	0.98 ± 0.11	0.98 ± 0.17	0.98 ± 0.12	1.01 ± 0.08	1.07 ± 0.10	1.09 ± 0.11	1.09 ± 0.10
HR	LS	101.20 ± 30.29	145.60 ± 23.02	164.60 ± 25.29	179.60 ± 12.78	189.80 ± 12.62	196.80 ± 11.17	191.60 ± 19.73	210.60 ± 7.44	213.00 ± 6.00
	MS	101.80 ± 37.56	138.80 ± 25.45	161.80 ± 22.60	169.80 ± 35.79	184.20 ± 26.58	197.80 ± 12.77	206.80 ± 10.76	211.80 ± 10.89	217.20 ± 6.30
	HS	98.60 ± 23.06	142.60 ± 26.13	161.00 ± 16.85	187.20 ± 17.56	191.60 ± 17.66	200.00 ± 15.03	205.80 ± 12.13	212.20 ± 10.26	216.40 ± 8.05

Heart rates

Heart rates during IET did not differ with treatment (Figure 12). Mean heart rates are presented in Table 8.

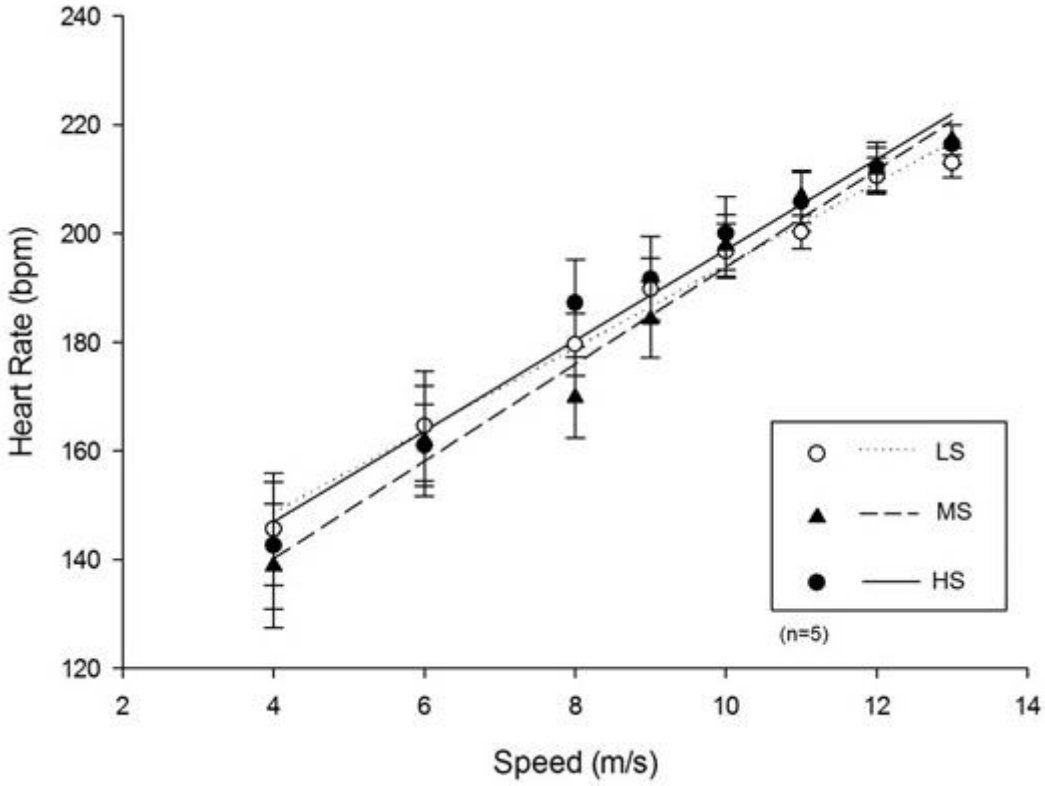

Figure 12. Heart rates during IET test. Values are Mean±SE for the low starch (LS), medium starch (MS) and high starch (HS) treatments.

RER (Respiratory Exchange Ratio)

To verify if substrate utilization was altered by depletion, RER was determined both before and immediately after the depletion protocol. RER obtained post depletion was different from pre-depletion for both HS and MS treatments ($p<0.05$). Furthermore, pre-depletion RER for the LS treatment differed from HS and MS ($p<0.05$), but was not

significantly different from post-depletion within treatment (Figure 13). Mean RER during the aerobic tests (Pre and Post ART) are presented in Table 9.

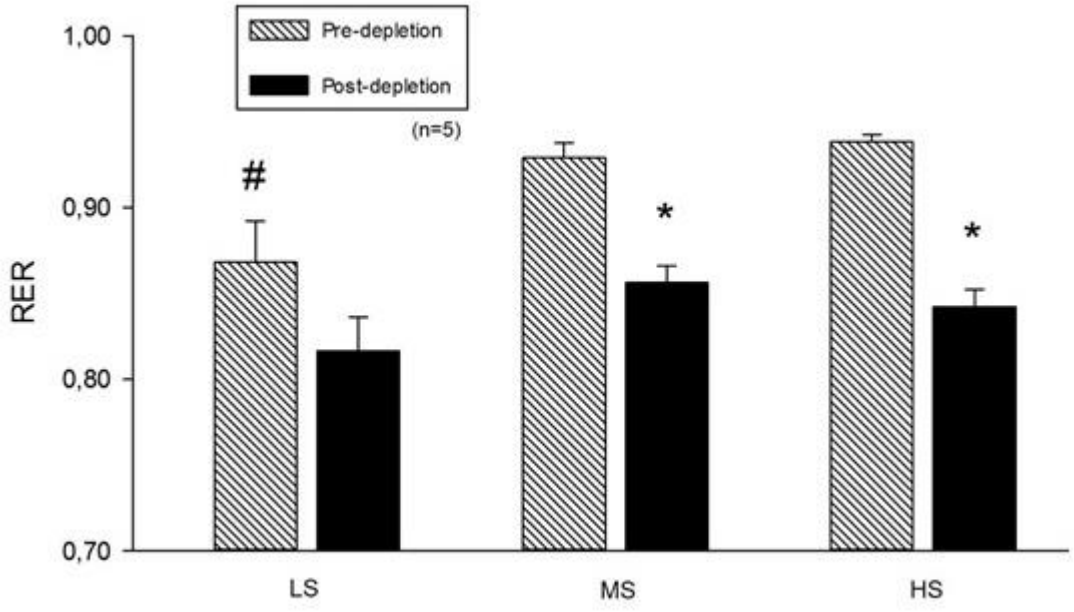

Figure 13. Respiratory Exchange Ratios (RER) during pre- and post-depletion aerobic tests. (*) Indicates difference from pre-ART within treatment ($p<0.05$).(#) Indicates difference from HS and MS treatments during pre-ART ($p<0.05$).

Table 9. Respiratory Exchange Ratios (RER) during pre-depletion aerobic test (Pre-ART) and post-depletion aerobic test (Post-ART). Values are Means±SD for LS (low starch), MS (medium starch) and HS (high starch) treatments.

	LS	MS	HS
PRE ART	$0.868^{bA} \pm 0.053$	$0.929^{aA} \pm 0.020$	$0.938^{aA} \pm 0.010$
POST ART	$0.816^{aA} \pm 0.045$	$0.856^{aB} \pm 0.022$	$0.842^{aB} \pm 0.023$

Different letters (lower case) in the same line indicate difference among treatments.
Different capital letters in the same column indicate difference between pre and post depletion within treatment.

RER was also measured during the high-intensity IET test (Figure 14). RER during the IETs did not significantly differ with treatment ($p>0.05$).

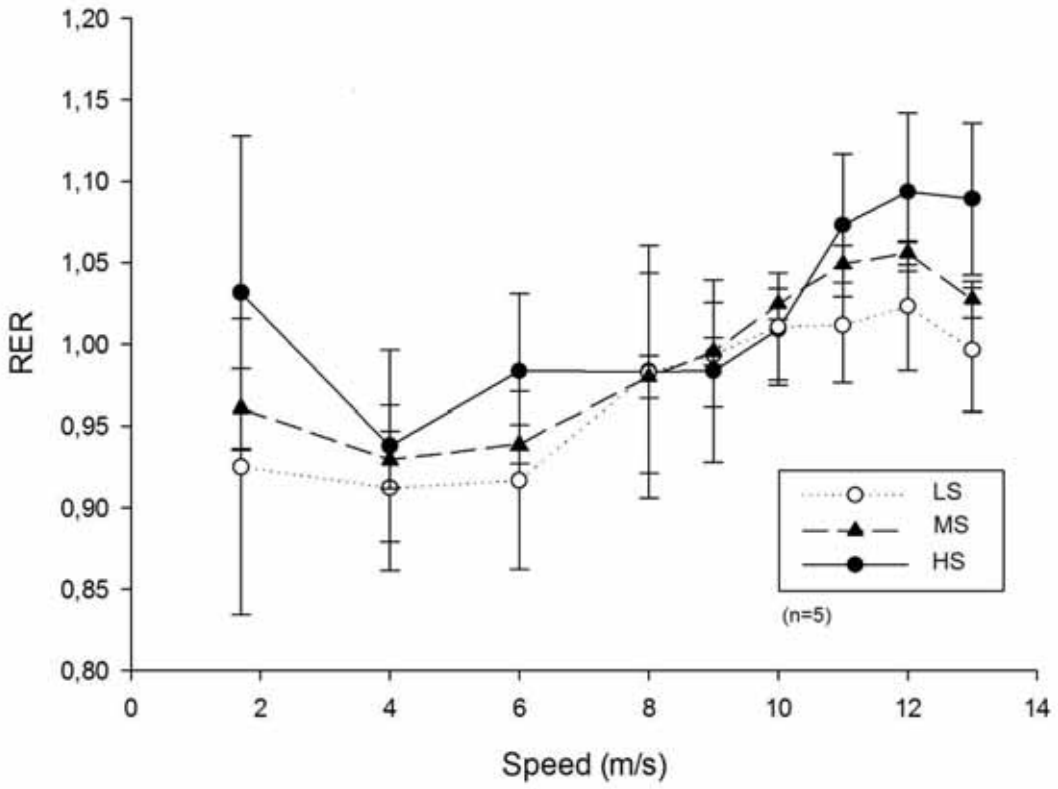

Figure 14. RER during IET. Figures represents Means±SE for the different speeds during the IET.

Muscle glycogen content

In order to assess whether the different treatments provided enough substrate for glycogen maintenance during training, muscle biopsies were obtained at the end of the three-week training period. Glycogen content at this point of the trial did not present significant changes for any treatments, and all three diets allowed for realization of the training regime and for maintenance of body weights. Muscle biopsies were also obtained after the three-day depletion protocol and every 24 hours after the end of depletion, for 72 hours. Values for glycogen concentrations before and after depletion and during the 72-hour recovery period are presented in Table 10. Post-training glycogen concentrations were 117.42±18.69, 119.81±10.59, and 121.95±10.05 mmol/kg wet

weight for HS, LS and MS treatments, respectively, and did not differ from one another ($p>0.05$). Depletion was also not different for any of the treatments, and had a mean±SD of 32.74±16.97, 29.7±7.55 and 36.5±14.47 for HS, LS and MS respectively. Depletion percentages are presented in Figure 15.

Table 10. Mean muscle glycogen concentrations (mmol/kg wet weight). Values are means ± SD for low starch (LS), medium starch (MS) and high starch (HS) treatments. (n=5)

	LS			MS			HS		
Pre-depletion	119.81	±	10.59	121.95	±	10.05	117.42	±	18.69
Post-depletion	83.94	±	8.99	76.84+	±	16.47	76.57*	±	7.20
24 hours	95.02	±	15.71	77.27+	±	15.06	90.23	±	11.29
48 hours	87.28	±	25.29	103.06	±	30.88	102.06	±	16.88
72 hours	76.49#	±	27.99	114.26	±	14.3	110.84	±	25.59

*Indicates difference from pre-depletion ($p<0.05$) within HS treatment.
#Indicates difference from pre-depletion ($p<0.05$) within LS treatment.
+Indicates difference from pre-depletion ($p<0.05$) within MS treatment.

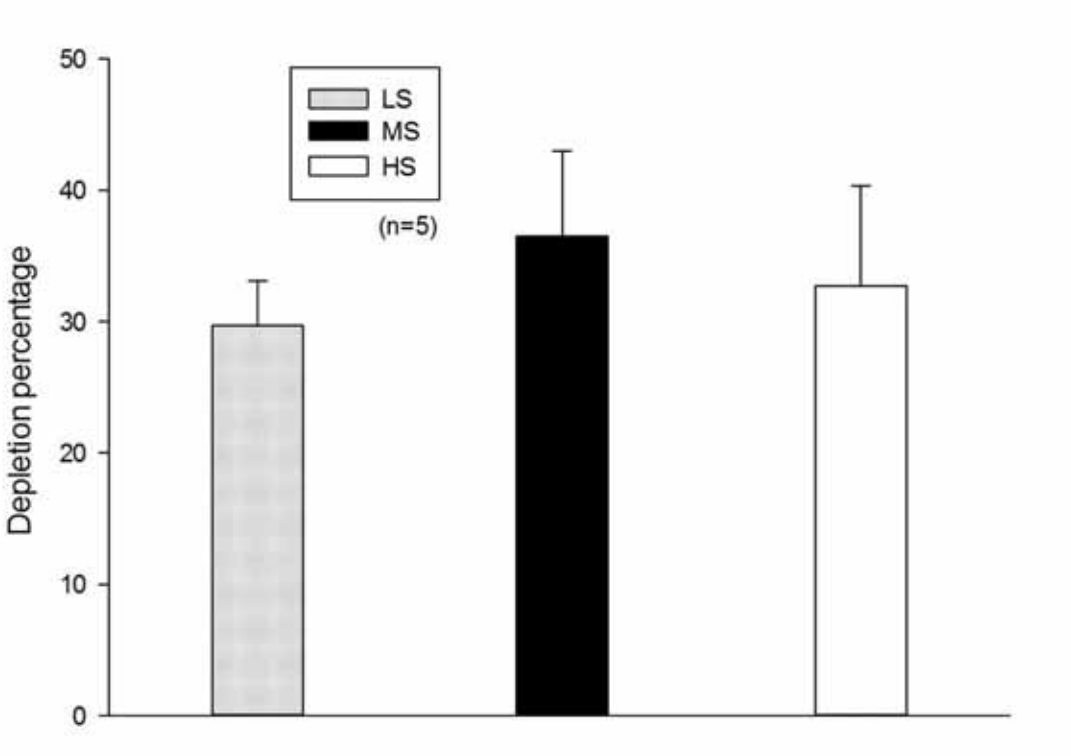

Figure 15. Mean (±SE) percentage of depletion caused by the depletion protocol on high (HS), medium (MS) and low starch (LS) diets.

Muscle Glycogen Repletion

Repletion of glycogen was similar for the HS and MS treatments ($p>0.05$). Repletion in the LS treatment, however, was significantly lower ($p<0.05$). At the end of 72 hours post-depletion (recovery period), horses on the low-starch diet (LS) had reached only 63.4% of pre-depletion values, while HS and MS horses returned glycogen stores to 94.3 and 94.1%, respectively. Furthermore, glycogen content in that group seemed to decrease even further during the repletion period, with concentrations of 83.94±9.00 (mean±SD), 95.02±15.71, 87.28±25.29 and 76.49±28.00 mmol/kg wet wt. at 0, 24, 48 and 72 hours post-depletion, respectively. Depletion and repletion percentages are represented in Figure 16.

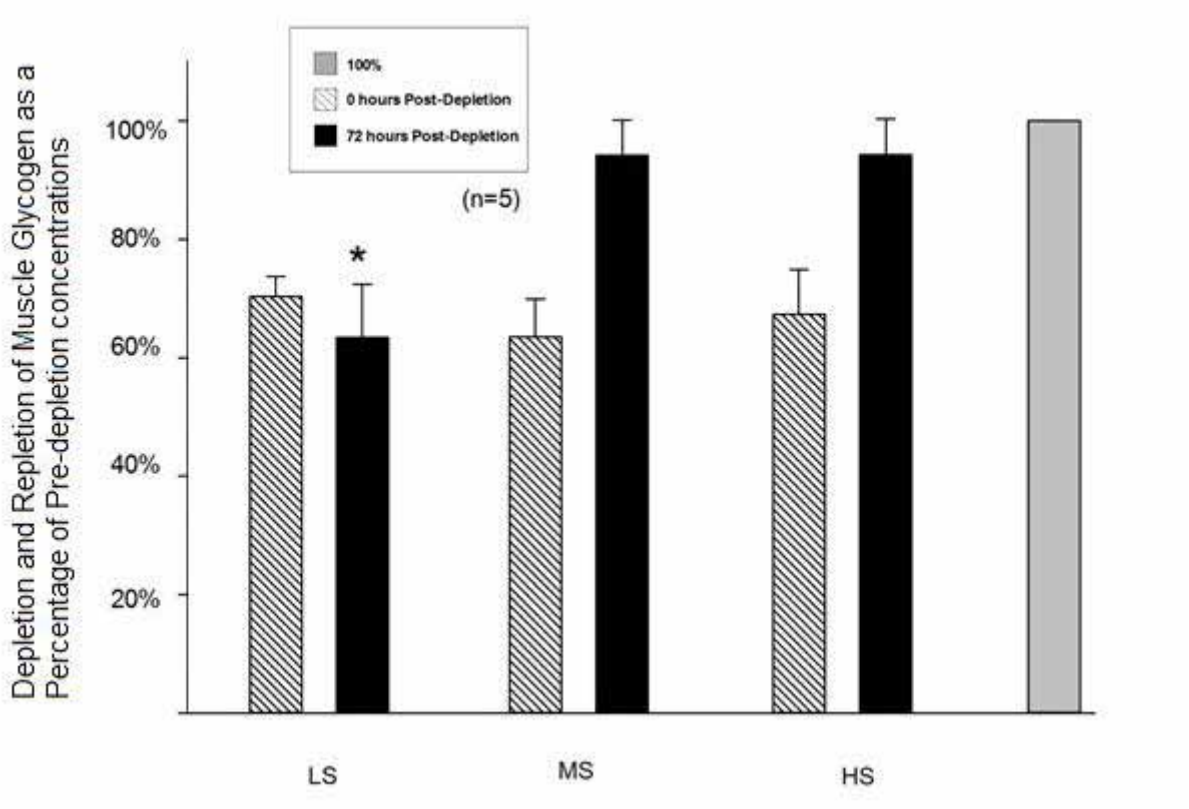

Figure 16. Muscle Glycogen after depletion (0 hours) and at 72 hours post-depletion for the low starch (LS), medium starch (MS) and high-starch (HS) treatments, as a percentage of pre-depletion values. (*) Indicates difference from HS and MS at 72 hours post-depletion ($p<0.05$).

6. DISCUSSION

Concentrates low in starch and high in fat and fiber have become increasingly popular amongst horse owners and riders in the Unites States and in Europe, and a large number of horses competing in equestrian sports such as three-day eventing and jumping consume this type of concentrate. This study indicates that following a substantially glycogen-depleting exercise, such as the cross-country step in a three-day event competition, horses on this type of diet may not replenish their glycogen reserves satisfactorily. Horses normally take 72 hours to fully restore glycogen reserves (HYYPPÄ; RASANEN; POSO, 1997; LACOMBE et al., 2004; SNOW et al., 1987).

Sports like three-day eventing, in which horses compete over three days, pose a challenge to the performance of the animals, once glycogen stores keep being drawn upon and there is not enough time for complete replenishment. A diet that does not favor glycogen replenishment would certainly enhance this problem. In this study, horses that underwent a glycogen depleting exercise protocol and consumed a concentrate low in starch and high in fat and fiber (LS) did not replenish glycogen stores as efficiently as horses on a traditional, high-starch diet concentrate (HS) or a concentrate composed of a high-starch, high fat corn ingredient (MS). These results agree with those of Lacombe et al. (2004), who found that a high-soluble carbohydrate diet hastened muscle glycogen resynthesis in horses after strenuous exercise that depleted at least 60% of initial glycogen reserves, compared to a mixed and a low-soluble carbohydrate diet. However, in that study diets were not isocaloric. Moreover, the amount of soluble carbohydrate used was such as could generate high risk of metabolic diseases.

In the present study, horses fed the low-starch diet (LS) not only had lower muscle glycogen after 72 hours of recovery, but their glycogen stores failed to return to pre-depletion levels, reaching only 63.4% of their initial values. Previous work has proven that horses are not very efficient at replenishing glycogen stores after intense exercise. In a study by Hyyppä, Rasanen and Poso (1997), horses underwent glycogen-depleting exercise and then were monitored for glycogen resynthesis during 72 hours post-exercise. Results showed that glycogen actually decreased during the initial 4 hours of

recovery and resynthesis was negligible within the first 24 hours post-depletion. Earlier studies also report a lack of resynthesis in the early stages of recovery (DAVIE; EVANS; HODGSON, 1995; SIMMONS; FORD, 1991; SNOW et al., 1982). Horses in the present study who were on the LS diet presented unsatisfactory repletion after 72 hours of recovery. One of the reasons for this might be related to the lower ($p<0.05$) glycemic index (GI) of this diet (31.75±6.33 GI) compared to the traditional, high-soluble carbohydrate (HS) control diet (134.00±13.99 GI) and the moderate starch and fat MS diet (92.25±23.77 GI). The lower GI would most likely have produced lower insulin and therefore may have negatively influenced glucose uptake by the muscle. Moreover, during recovery from exercise, blood glucose is the only source available for glycogen resynthesis (HINCHCLIFF;GEOR;KANEPS, 2008), either through liver glycogenolysis or gastrointestinal absorption of glucose from the diet. A diet poor in this nutrient could fail to provide enough substrate for glycogenesis, as shown by previous studies (ESSEN-GUSTAVSSON et al., 1989; HYYPPÄ; RASANEN; POSO, 1997). Liver glycogen depletion may also have been a contributing factor; however, it was not possible to assess liver glycogen content in this study.

Recent research by Vonderohe et al. (2014) further confirms that low-starch diets do not promote adequate glycogen replenishment. These researchers fed Quarter horses either a low or high-starch commercial concentrate at 0.75% kg BW/d, plus 1% kg BW/d of grass hay, with diets providing 997.6 g of starch and 553.7 g of starch/d respectively. After a depleting exercise test, they found that horses on the low-starch diet replenished glycogen at a slower rate than horses on the high-starch diet, and that at the end of 48 hours post-depletion the low-starch diet produced lower glycogen concentration than the high-starch diet ($p<0.05$). These results are in agreement with the findings in the present study, in which three concentrates with varying levels of starch were tested. The LS treatment provided 392.3±29 g of starch/d, while the MS and HS treatments provided 2,155.5±159.6 and 3,115.2±230.6 g of starch/d each, respectively.

Results in the present study suggest that in situations where glycogen depletion is greater than 30%, a strategy must be developed to allow horses on this type of concentrate to adequately replenish glycogen stores. Such strategies may include the administration of glucose, either orally or intravenously, both of which have been

investigated previously. Davie, Evans and Hodgson (1995) provided horses intravenously with 6g/kg BW of dextrose after depletion of approximately 48% of glycogen reserves and found that dextrose infusion hastened glycogen resynthesis. Similarly, Geor et al. (2006) provided glucose supplementation both intravenously and orally after exercise that produced approximately 50% glycogen depletion. They observed higher plasma glucose and serum insulin for both glucose treatments as compared to control (no glucose added), and also observed faster rates of glycogenesis in the initial 6 hours of recovery for horses supplemented intravenously, but not orally, with glucose. In another study, Lacombe et al. (2001) infused horses depleted of approximately 55% of their initial glycogen with either a glucose or a saline solution. Rates of glycogen replenishment were greater for the glucose treatment. However, intravenous administration of glucose are invasive and might even be prohibited at some competitions.

Concentrates with lower starch content have been largely used to manage muscular disorders such as recurrent exertional rhabdomyolysis and polysaccharide storage myopathy. Ribeiro et al. (2004) studied the effect of diets with varying starch and fat contents on horses suffering from polysaccharide storage myopathy and found that diets with less than 5% of Digestible Energy (DE) contribution from starch and more than 12% of DE coming from fat can potentially diminish exertional rhabdomyolysis. McKenzie et al. (2003) also evaluated the effect of a low starch (7% DE from starch) and high fat (20% DE from starch), compared to a high starch (40% of DE), low fat (5% of DE) diet and an identical diet to the high starch but supplemented with bicarbonate on horses severely affected by recurrent exertional rhabdomyolysis. The low starch, high fat diet produced much lower CK activity in affected horses. Success at managing muscular and metabolic disorders by feeding low starch, high fat diets in addition to wide knowledge of the risks involved in feeding high amounts of starch created a trend towards concentrates low in carbohydrates and high in fat. Commercial concentrates available in the market typically contain high fat inclusion and are comprised greatly of fermentable fibers, more traditionally beet pulp.

Both the inclusion of fat to the athlete horse's diet and the replacement of starch with highly fermentable fiber have been investigated as an alternative to provide energy without the risks of a high starch diet.

Addition of fat has been shown to promote better performance through higher glycogen concentrations (HARKINS et al., 1993; MEYERS et al., 1989; OLDHAM et al., 1990; SCOTT et al., 1992). This was suggested to be the product of a glycogen-sparing effect, with the horse utilizing more fat as an energy substrate and thus drawing from glycogen stores later than they would have without the added fat. High-fat diets also promoted a shift towards lipid oxidation as an energetic pathway in preference to CHO metabolism in horses performing low, moderate and intense exercise (DUNNET; MARLIN; HARRIS, 2002; DUREN et al., 1987; HAMBLETON et al., 1980; HARKINS et al., 1993; HINTZ et al., 1987; OLDHAM et al., 1990)

The effects of including highly fermentable fibers in the equine athlete's diet, such as beet pulp and soy hulls, have also been studied. Palmgren Karlsson et al. (2002) tested the partial replacement of oats with molassed sugar beet pulp. Horses on the molassed super beet pulp diet had lower lactates and higher glycogen content in the muscle after exercise, indicating a sparing effect of glycogen reserves. Similarly, Jansson et al. (2002) investigated the effects of substitution of oats for barley sugar, and found that it promoted lower glycogen utilization, suggesting a glycogen-sparing effect. In another study, Lindberg and Palmgren Karlsson (2001) substituted oats by plain sugar beet pulp and found reduced post-prandial glycemic and insulinemic responses. In the present study, a concentrate with low starch and high inclusion of fat and fermentable fibers (LS) did not promote adequate glycogen replenishment after a substantially glycogen-depleting exercise test. However, during the training portion of the trial, horses on the LS diet were able to perform their daily training routine without adverse effects, and did not significantly differ from horses consuming the HS and MS concentrates when post-training glycogen levels were compared. This shows that the LS concentrate allowed horses to maintain glycogen stores throughout a daily training regime as well as horses on traditionally high-starch concentrates. Ability to maintain muscle glycogen during daily training by all diets was demonstrated by the muscle glycogen content at the end of the training portion of the trial, right before the beginning of the depletion protocol, which did not differ with treatment. Possibly, the depletion level of the training protocol was not very high and all treatments were able to keep glycogen reserves replenished on a day-to-day basis. Snow and Harris (1991) found that horses being trained for flat racing

depleted about 19 to 25% of their glycogen reserves after galloping exercises of 1000 and 1600 meters and restored glycogen levels within 2-3 days. In a similar study, Nimmo and Snow (1983) found Thoroughbred racehorses in training to deplete their glycogen by 28% at distances of 506, 1025, 1600 and 3620 meters. In the present study, horses covered on average 1.15±0.01 meters at the gallop (10m/s), in addition to another 2.82±0.03 at a canter (8m/s). Both Nimmo and Snow (1983) and Snow and Harris (1991) studies' weekly training protocols included full-out gallops that most likely exceeded 10m/s. The top speeds used in the treadmill workouts in the present study did not reach full-out gallops, which combined with the lack of inclination most likely did not promote great depletion, and would account for horses being able to maintain glycogen during training on the LS diet as efficiently as the other diets. Moreover, exercise on the walker, interspaced with the treadmill workouts, was predominantly aerobic, and thus would have used mainly lipid and glucose oxidative phosphorylation, possibly sparing further glycogen depletion and allowing some repletion to take place in between bouts of treadmill work. To verify the depletion power of everyday exercise, muscle biopsies would have to have been taken during the training period; however, in this trial muscle biopsies were only sampled prior to and after the depletion protocol and during the 72-hour recovery period. Nevertheless, these findings indicate that concentrates with low starch content are adequate for daily training that does not substantially deplete glycogen reserves.

In the present study, horses consuming the LS concentrate did have lower RER during the aerobic tests, suggesting there was greater fat contribution to energy production during submaximal exercise. However, horses on the LS concentrate did not present higher glycogen content at the end of the depleting protocol. More importantly, they did not show significant differences in regards to pre-depletion glycogen levels. At the end of each three-week training period there were no differences in glycogen concentrations among treatments, indicating there was no perceivable glycogen-sparing effect during the training period. LS, MS and HS concentrates had 14, 8 and 5% fat content respectively, and total diets for each of the respective treatments were 23, 14 and 11% in fat. Several studies have demonstrated that the addition of fat to the diet of performance horses alters substrate utilization (GEELEN et al., 2001; KOHNKE, KELLEHER; TREVOR-JONES, 1999; KRONFELD et al., 1994; ORME et al 1997).

Authors of these studies have found the addition of fat to promote a glycogen sparing effect, which was not observed in our study. The lack of a sparing effect during the training portion of the trial could be due to the fact that the work performed by the horses was not sufficiently glycogen depleting. However, previous research found that providing more than 15% of the total diet as fat resulted in lower glycogen concentrations (PAGAN et al., 1987), while others found no change in glycogen with the addition of fat to the diet (GREIWE et al., 1989; EATON et al., 1995). The LS diet in this study had a fat content of 23% in total diet, and that could have accounted for the lower glycogen concentrations produced by this treatment.

Findings in the present study demonstrated that horses were able to adapt to the high-fat diet within the three-week training portion of the trial, as demonstrated by lower RER in the LS treatment, both in the pre-depletion submaximal test and in the IET on the first day of the depletion protocol. This observation suggests that three weeks may be sufficient for horses to adjust to dietary fat supplementation and shift their energy metabolism from carbohydrate to fat utilization. A five-week adaptation period has been previously suggested by Custalow et al.(1993) and verified by other studies (DUNNET; MARLIN; HARRIS, 2002; MARCHELLO et al., 2000; PAGAN et al., 2002;). However, Hyyppä, Saastamoinen and Poso (1999) found that horses consuming a fat-supplemented diet for three weeks showed changes compatible with fat metabolism adaptation, even though fat was only 5% of total DM. This was also observed in a study by Orme et al. (1997), who reported differences in metabolic responses associated with a fat-supplemented diet after three weeks of fat supplementation, indicating that a shift towards lipid metabolism occurs before five weeks of fat supplementation.

The lower RER in the LS treatment during the pre-depletion submaximal test clearly show that horses on a high-fat diet have higher efficiency at obtaining energy from lipid metabolism. This in accordance with previous research, which found lower respiratory quotients to be associated with dietary fat adaptation. Dunnet, Marlin and Harris (2002) found lower RER after supplementing fat to aerobically trained Thoroughbred horses for 5 and 10 weeks. Moreover, they also found that reverting fat-adapted horses to a control diet with low fat produced higher RER. Pagan et al. (2002) reported lower RER in horses after 5 and 10 weeks of fat supplementation (10% fat in

total ration on an as fed basis) in Arabian horses during a 90 minute treadmill test at 35% VO_{2max} when compared to horses fed a low fat (2.6% total ration, as fed basis) control diet. Although NEFA was not measured, the lower RER found in LS treatment is probably due to higher capacity to utilize NEFA from circulating triacylglycerol (DUNNET; MARLIN; HARRIS, 2002; GEELEN et al., 2000; ORME et al., 1997; PAGAN et al., 2002).

Interestingly, horses on the high and medium starch treatments (HS and MS) demonstrated lower RER during the post-depletion submaximal test. This is evidence that horses shift their energy metabolism according to substrate availability and that substrate depletion, in this case glycogen, limits its efficiency. In this study, when horses were depleted of glycogen by more than 30%, subsequent aerobic exercise produced lower RER than the same type of exercise before depletion for horses on diets with high (HS) and medium (MS) starch content.

Lactate means during the IET for the LS diet were lower than for HS or MS. These observations agree with those of Meyers et al. (1989), who found a trend ($p<0.16$) for lower lactate in horses supplemented with fat and subjected to a submaximal exercise test on the treadmill. In another study, Greiwe et al. (1989) also found lower lactate associated with fat supplementation. Sloet Van Old Ruitenborgh-Oosterbaan et al. (2002) also observed lower lactates ($p=0.011$) in Standardbreds fed a high fat diet (11.8% total diet DM) and subjected to a submaximal standardized stepwise exercise test on the treadmill, compared to a low-fat diet (1.5% total diet DM). Conversely, several other researchers found higher lactates associated with a high-fat diet. Custalow et al. (1993) sprint-trained Arabians fed a diet supplemented with 10% corn oil and found higher blood lactate concentrations during incremental exercise tests on a high-speed treadmill. In a similar study (FERRANTE et al., 1993), horses adapted to a high-fat diet had higher lactates during repeated sprints on the treadmill. Webb et al. (1987) also reported higher blood lactate following intense exercise in horses supplemented with fat, as well as other researchers (PAGAN et al., 1993; TAYLOR et al., 1995). The differences in these observations might be related to the type of training and the changes in energetic metabolism elicited by aerobic (endurance) training and anaerobic (sprint) training, as suggested by Kronfeld et al. (1994). Lactate is the end product of anaerobic glycolysis (HINCHCLIFF; GEOR; KANEPS, 2008), and lower blood lactate levels could indicate that

horses in the LS treatment utilized less glycogen during the IET, although no sparing effect was observed at the end of the depletion three-day protocol in this study. Even though mean lactates were lower for the LS treatment, there was no difference among treatments in VLa2 and VLa4, which is probably related to the low number of horses in the trial.

Although HS and MS diets proved more efficient than LS at replenishing muscle glycogen, LS was perfectly capable of sustaining glycogen reserves throughout the training period. In practical terms, the MS diet might be the best alternative for horses competing at sports that substantially deplete glycogen, because it would provide sufficient substrate for glycogen replenishment at a lower risk than a traditional high-starch diet, and would also avoid problems related to an abrupt switch from a low-starch to a starch-rich diet during recovery, which would be the case should the horse be on a low-starch diet and then be fed a starch-rich diet or supplement in an attempt to hasten glycogen resynthesis.

7. CONCLUSIONS

Low soluble-carbohydrate, high-fat diets provided enough substrate to maintain glycogen levels during the training period of the trial, which simulated a regime likely to be seen in horses being daily trained for eventing, show-jumping, initial racing and other common equestrian sports.

However, this study shows that diets with low soluble-carbohydrate content do not allow for proper glycogen replenishment after substantial (>30%) depletion has taken place.

Aerobic exercise tests confirmed that horses on a high fat, high-fiber diet do favor lipolysis as an energy pathway during sub-maximal exercise. Furthermore, this study demonstrates that previous substantial depletion of glycogen reserves promote a shift towards lipid metabolism in horses, independent of the amount of starch or fat in the diet.

In summary, diets with very low starch content appear to be adequate for daily training that does not deplete glycogen reserves greatly and relies predominantly on aerobic energy pathways. However, after highly intense exercise bouts, which promote substantial depletion, feeding carbohydrates seems to be essential for muscle glycogen resynthesis, and commercial “low starch” feeds do not seem to provide enough substrate.

Implications of the present study indicate that a moderate-starch diet may be a more appropriate solution to glycogen maintenance and replenishment after substantial depletion.

8. REFERENCES

BRÖJER, J.; HOLM, S.; JONASSON, R.; HEDENSTRÖM, U.; ESSÉN-GUSTAVSSON, B. Synthesis of proglycogen and macroglycogen in skeletal muscle of Standardbred trotters after intermittent exercise. **Equine Veterinary Journal**, London, v.38, p. 335–339, 2006. DOI: 10.1111/j.2042-3306.2006.tb05564.x

COSTILL, D. L.; SHERMAN, W. M.;FINK, W. J.; MARESH, C.;WITTEN,M.; MILLER, J. M. The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. **American Journal of Clinical Nutrition**, New york, v. 34, p.1831-1836, 1981.

CUSTALOW, S. E.; FERRANTE, P. L.; TAYLOR, L. E.; MOLL, H. D.; MEACHAM, T. N.; KRONFELD, D. S.; TIEGS, W. Lactate and glucose responses to exercise in the horse are affected by training and dietary fat. **Proceedings of Equine Nutrition and Physiology Society,** v.13, p. 179-184, 1993.

DAVIE, A.J.; EVANS, D.L.; HODGSON D.R. Effects of intravenous dextrose infusion on muscle glycogen resynthesis after intense exercise. **Equine Veterinary Journal,** London, v.18, p. 195-198, 1995.

DAVIE, A.J.; EVANS, D.L.; HODGSON, D.R.; ROSE R.J. Effects of glycogen depletion on high intensity exercise performance and glycogen utilization rates. **Pferdehelkunde,** Sonderheft, v.12, p 482-484, 1996.

DAVIE, A.J.; EVANS, D. L.; HODGSON, D. R.; ROSE R. J. Glycogen depletion and repletion in the horse – possible limiting factor in performance (Review). In: RALSTON, S. L. **Advances in equine nutrition.** Nottingham University Press, 2001. v.2, p. 509-518, 2001.

DOREAU, M.; BOULOT, S.; BAUCHART, D.; BARLET, J. P.; MARTIN-ROSSET, W. Voluntary intake, milk production and plasma metabolites in nursing mares fed two different diets. **The Journal of Nutrition**, v. 122, n. 4, p. 992-999, 1992.

DUNNETT, C.E., MARLIN, D.J., HARRIS, R.C. Effect of dietary lipid on response to exercise: relationship to metabolic adaptation. **Equine Veterinary Journal**, London, v.34, p.75-80, 2002. Supplement

DUREN S.E.; JACKSON S.G.; BAKER J.P.; AARON, D.K. **Effect of dietary fat on blood parameters in exercised thoroughbred horses**. Cambridge: ICEEP, 1987. p. 674-685.

EATON, M.D. Energetics and performance. In: HODGSON, D.R.; ROSE, R.J. **The athletic horse**: principles and practice of equine sports medicine. Philadelphia: Saunders, 1994. p. 49-62.

EATON, M.D.; HODGSON, D.R.; EVANS, D.L. Effect of diet containing supplementary fat on the capacity for high-intensity exercise. **Equine Veterinary Journal**, London, v.18, p. 353-356, 1995. Supplement.

ESSÉN-GUSTAVSSON, B.; MCMIKEN, D.; KARLSTRÖM, K.; LINDHOLM, A.; PERSSON, S.; THORNTON, J. Muscular adaptation of horses during intensive training and detraining. **Equine Veterinary Journal**, London, v. 21, n.1, p. 27-33, 1989. DOI: 10.1111/j.2042-3306.1989.tb02085.x

ESSÉN-GUSTAVSSON, B.; BLOMSTRAND, E.; KARLSTRÖM, K.; LINDHOLM, A.; PERSSON, S.G.B. Influence of diet on substrate metabolism during exercise. In: PERSSON, S.G.B.; LINDHOLM, A.; JEFFCOTT, L.B. **Equine exercise physiology 3.** Davis: ICEEP, 1991. p. 288-298.

FERRANTE, P. L.; TAYLOR, L. E.; MEACHAM, T. N.; KRONFELD, D. S.; TIEGS, W. Evaluation of acid-base status and strong ion difference (SID) in exercising horses. **Proceedings of Equine Nutrition and Physiology Society**, v.13, p.123-124, 1993.

GARNER, H.E.; COFFMAN, J.R.; HAHN, A.W.; SALEM, C. Equine laminitis of alimentary origin: an experimental model. **American Journal of Veterinary Research**, Chicago, v. 36, p. 441-445, 1975.

GEELEN, S.N.J.; JANSEN, W.L.; GEELEN, M.J.H.; SLOET VAN OLDRUITENBORGH-OOSTERBAAN, M.M.; BEYNEN, A.C. Lipid metabolism in equines fed a fat-rich diet. **International Journal for Vitamin and Nutrition Research.** v.70, p.148-152, 2000.

GEELEN, S.N.; BLÁSQUEZ, C.; GEELEN, M.J.; SLOET VAN OLDRUITENBORGH-OOSTERBAAN, M.; BEYNEN, A.C. High fat intake lowers hepatic fatty acid synthesis and raises fatty acid oxidation in aerobic muscle in Shetland ponies. **British Journal of Nutrition**, Cambridge, v.86, p.31-36, 2001. DOI: 10.1079/BJN2001364

GEOR, R.J. Role of dietary energy source in the expression of chronic exertional myopathies in horses**. Journal of Animal Science**, Champaign, v.83, p. 32-36, 2005.

GEOR, R.J.; LARSEN, L.; WATERFALL, H.L.; STEWART-HUNT, L.; MCCUTCHEON, L.J. Route of carbohydrate administration affects early post

exercise muscle glycogen storage in horses. **Equine Veterinary Journal,** London, v.36, p. 590-595, 2006. Supplement.

GREIWE, K.M; MEACHAM,T.N.; FREGIN,G.F.; WALBERG, J.L. Effect of added dietary fat on exercising horses. In**:** EQUINE NUTRITION AND PHYSIOLOGY SYMPOSIUM, 11., 1989, Stillwater. **Proceedings**…

HAMBLETON, P.L.; SLADE, L.M.; HAMAR,D.W.; KIENHOLZ,E.W.; LEWIS, L. D. Dietary Fat and Exercise conditioning effect on metabolic parameters in the horse. **Journal of Animal Science**, Champaign, v. 51, n.6, p.1330-1339, 1980.

HARKINS, J.D.; MORRIS, G.S.; TULLEY, R.T.; NELSON, A.G.; KAMERLING, S.G. Effect pf added dietary fat on racing performance in Thoroughbred horses. **Equine Veterinary Science**, London, v.12, p. 123-129, 1993.

HARRIS, P.A. Energy sources and requirements of the exercising horse. **Annual review of nutrition.** v.17, p.185-210, 1997.

HARRIS, P.A. Comparison of digestible energy (DE) and net energy (NE). **Advances in equine nutrition.** Nottingham University Press, 1999. v.2, p. 199-213.

HINCHCLIFF, K.W.; KANEPS, A.J.; GEOR, R.J. (Ed.). **Equine exercise physiology**: the science of exercise in the athletic horse**.** Elsevier Health Sciences, 2008. p. 247-273.

HINTZ, H.F.; ROSS, M.W.; LESSER, F.R.; LEIDS, P.F.; WHITE, K.K.; LOWE, J.E.; SHORT, C.E.; SCHRYVER,H.F. The value of dietary fat for working horses: biochemical and hematological evaluations. In: EQUINE NUTRITION AND PHYSIOLOGY SYMPOSIUM, 10., 1987, Fort Collins. **Proceedings**…p. 483-488.

HODGSON, D.R.; ROSE, R.J.; DIMAURO, J.; ALLEN, J.R. Muscle glycogen depletion and repletion pattern in horses performing various distances of endurance exercise. In: SNOW, D.H.; PERSSON, S.G.B.; ROSE, R.J (Ed.). **Equine exercise physiology**. Cambridge: Granta Editions, 1983. p. 229-236.

HODGSON, D.R.; ROSE, R.J.; ALLEN, J.R.; DIMAURO, J**.** Glycogen depletion patterns in horses competing in day 2 of a three-day event. **Cornell Veterinarian**, Ithaca, v.75, p. 366–374, 1984.

HODGSON, D. R.; ROSE, R. J.; DIMAURO, J.; ALLEN, J. R. Effects of a submaximal treadmill training programme on histochemical properties, enzyme

activities, and glycogen utilization of skeletal muscle in the horse. **Equine Veterinary Journal**, London, v. 17, p. 300-305, 1985.

HODGSON, D.R.; ROSE, R.J.; DIMAURO, J.; ALLEN, J.R. Effects of training on muscle composition in horse. **American Journal of Veterinary Research**, Chicago, v.47, p. 12-15, 1986.

HOFFMAN, R.M.; WILSON, J.A.; KRONFELD, D.S.; COOPER, W.L.; LAWRENCE, L.A.; SKLAN, D.; HARRIS, P.A. Hydrolysable carbohydrates in pasture, hay, and horse feeds: direct assay and seasonal variation. **Journal of Animal Science**, Champaign, v.79, p. 500-506, 2001.

HOFFMAN, R.M.; BOSTON, R.C.; STEFANOVSKI, D.; KRONFELD, D.S.; HARRIS, P.A. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. **Journal of Animal Science**, Champaign, v.81, p. 2333-2342, 2003.

HYYPPÄ, S.; RASANEN, L.A.; POSO, A.R. Resynthesis of glycogen in skeletal muscle from Standardbred trotters after repeated bouts of exercise. **American Journal of Veterinary Research**, Chicago, v.58, p.162-166, 1997.

HYYPPÄ, S.; SAASTAMOINEN, M.; POSO, A.R. Effect of a post exercise fat-supplemented diet on muscle glycogen repletion. **Equine Veterinary Journal**, London, v.30, p. 493-498, 1999.

JANSSON, A.; NYMAN, S.; LINDHOLM, A.; LINDBERG, J.E. Effects on exercise metabolism of varying dietary starch and sugar proportions. **Equine Veterinary Journal**, London, **,**v.34, p.17-21, 2002. Supplement.

JENTJENS, R.L.P.G.; VANLOON, L.J.C.; MANN, C.H.; WAGENMAKERS, A.J.M.; JEUKENDRUP, A.E. Addition of protein and amino acids to carbohydrates does not enhance post-exercise muscle glycogen synthesis. **Journal of Applied Physiology**, Bethesda, v.91, p. 839-846, 2001.

JOSE-CUNILLERAS, E.;TAYLOR, L.E.; HINCHCLIFF, K.W. Glycemic index of cracked corn, oat groats and rolled barley in horses. **Journal of Animal Science**, Champaign, v.82, p. 2623-2629, 2004.

JOSE-CUNILLERAS, E.; HINCHCLIFF, K.W.; LACOMBE, V.A.; SAMS, R.A.; KOHN, C.W.; TAYLOR, L.E.; DEVOR, S.T. Ingestion of starch-rich meals after exercise increases glucose kinetics but fails to enhance muscle glycogen replenishment in horses. **The Veterinary Journal**, London, v.171, p.468-477, 2006.

KOHNKE, J. R.; KELLEHER, F.; TREVOR-JONES, P. In: **Feeding Horses in Australia**: a guide for horse owners and managers. Barton: Rural Industries Research & Development, 1999. p. 132-133.

KRONFELD, D.S.; FERRANTE, P.L.; GRANDJEAN, D. Optimal Nutrition for Athletic Performance, with Emphasis on Fat Adaptation in Dogs and Horses. **Journal of Nutrition**, Philadelphia, v.124, 12 Suppl., p. 2745-2753, 1994.

LACOMBE, V.; HINCHCLIFF, K.W.; GEOR, R.J.; LAUDERDALE, M.A. Exercise that induces substantial muscle glycogen depletion impairs subsequent anaerobic capacity. **Equine Veterinary Journal**, London, v.30, p. 293-297, 1999. Supplement.

LACOMBE, V.; HINCHCLIFF, K.W.; GEOR, R.J.; BASKIN, C.E. Muscle glycogen depletion and subsequent replenishment affect anaerobic capacity of horses. **Journal of Applied Physiology**, Bethesda, v.91, p. 1782-1790, 2001.

LACOMBE, V. A.; HINCHCLIFF, K. W.; TAYLOR, L. E. Interactions of substrate availability, exercise performance, and nutrition with muscle glycogen metabolism in horses. **Journal of the American Veterinary Medical Association**, Chicago,v. 223, n.11, p.1576-1585, 2003a.

LACOMBE, V.A.; HINCHCLIFF, K.W.; DEVOR, S.T. Effects of exercise and glucose administration on content of insulin-sensitive glucose transporter in equine skeletal muscle. **American Journal of Veterinary Research**, Chicago, v. 64, p.1500-1506, 2003b.

LACOMBE, V. A.; HINCHCLIFF, K. W.; KOHN, C. W.; DEVOR, S. T.; TAYLOR, L. E. Effects of feeding meals with various soluble-carbohydrate content on muscle glycogen synthesis after exercise in horses. **American Journal of Veterinary Research**, Chicago, v.65, p. 916–923, 2004. DOI: 10.2460/ajvr.2004.65.916

LINDBERG, J. E.; PALMGREN KARLSSON, C. Effect of partial replacement of oats with sugar beet pulp and maize oil on nutrient utilisation in horses. **Equine Veterinary Journal**, London, v.33, p. 585–590, 2001. DOI: 10.2746/042516401776563535

LINDHOLM, A.; PIEHL, K. Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscle of Standardbred horses. **Acta Veterinaria Scandinavica**, Kopenhagen, v.15, p. 287-309, 1974.

LOWRY, O.H.; PASSONNEAU, J.V. **A flexible system of enzymatic analysis**. New york: Academic Press, 1972.

MACLEAY, J.M.; VALBERG, S.J.; PAGAN, J.; BILLSTROM, J.A.; ROBERTS, J. Effect of diet and exercise intensity on serum CK activity in Thoroughbreds with recurrent exertional rhabdomyolysis. **American Journal of Veterinary Research**, Chicago, v.61, p.1390-1395, 2000.

MARCHELLO, E.V.; SCHURG, W.A.; MARCHELLO, J.A.; CUNEO, S.P. Changes in lipoprotein composition in horses fed a fat-supplemented diet. **Journal Of Equine Veterinary Science**, Wildomar, v.20, n.7, p.453-458, 2000.

MARLIN, D.; NANKERVIS, K. **Equine exercise physiology.** Oxford: Blackwell, 2002.

MARQUEZE, A.; KESSLER, A.M.; BERNARDI, M.L. Aumento do nível de óleo em dietas isoenergéticas para cavalos submetidos a exercício. **Ciência Rural**, Santa Maria, v.31, n.3, p.491-496, 2001.

MCKENZIE, E.C.; VALBERG, S.J.; GODDEN, S.M.; PAGAN, J.D.; MACLEAY, J.M.; GEOR, R.J.; CARLSON, G.P. Effect of dietary starch, fat, and bicarbonate content on exercise responses and serum creatine kinase activity in equine recurrent exertional rhabdomyolysis. **Journal of Veterinary Internal Medicine**, Tokyo, v.17, p.693–701, 2003.

MEYER, H.;RADICKE, S.;KIENZLE, E.;WILKE, S.;KLEFFKEN, D.;ILLENSEER, M. Investigations on preileal digestion of starch from grain, potato and manioc in horses. **Journal of Veterinary Medicine Series Association**, Lawrence, v.42, n.1-10, p. 371-381, 1995.

MEYERS, M.C.; POTTER, G.D.; EVANS, J.W.; GREENE, L.W.; CROUSE, S.F. Physiologic and metabolic response of exercising horses to added dietary fat. **Journal of Equine Veterinary Science**, Wildomar, v.9, p. 218-223, 1989. Available at: <http://doi:10.1016/S0737-0806(89)80056-5

MURRAY, M.J. Gastric ulcers in adult horses. **Comparative on Continuing Education Practising Veterinarian**, v. 16, p.792-797, 1994.

NIMMO, M. A.; SNOW, D. H. Changes in muscle glycogen, lactateand pyruvate concentrations in the thoroughbred horse following maximal exercise. In: SNOW, D.,PERSSON, S. G. B., ROSE, R. J. (Ed.). **Equine exercise physiology**. Cambridge: Granta Edition, 1983. p. 237-244.

NATIONAL RESEARCH COUNCIL. The Nutrient Requirements of Horses, 6th revised edition. National Academy Press, Washington DC, 2007.

OLDHAM, S.L.; POTTER, G.D.; EVANS, J.W.; SMITH, S.B.; TAYLOR, T.S.; BARNES, W.S. Storage and mobilization of muscle glycogen in exercising horses fed a fat-supplemented diet**. Journal of Equine Veterinary Science**, Wildomar, v.10, p. 353-355, 358-359, 1990. DOI:10.1016/S0737-0806(06)80096-1

ORME, C.E.; HARRIS, R.C.; MARLIN, D.J.; HURLEY, J. Metabolic adaptation to a fat-supplemented diet by the thoroughbred horse. **British Journal of Nutrition**, Cambridge, v.78, p. 443-458, 1997.

PAGAN, J.D.; ESSEN-GUSTAVSSON, B.; LINDHOLM, A.; THORNTON, J. The effect of dietary energy source on blood metabolites in Standardbred horses during exercise. In: EQUINE NUTRITION AND PHYSIOLOGY SOCIETY SYMPOSIUM, 10, 1987, Colorado State University. **Proceedings**… p. 425-430.

PAGAN, J.D.; ESSEN-GUSTAVSSON, B.; LINDHOLM, A.; THORNTON, J. The effect of exercise and diet on muscle and liver glycogen repletion in Standardbred horses. In: EQUINE NUTRITION AND PHYSIOLOGY SOCIETY SYMPOSIUM, 10, 1987, Colorado State University. **Proceedings**…p. 431-436.

PAGAN, J.D.; TEIGS, W.; JACKSON, S.G.; MURPHY, H.Q. The effect of different fat sources on exercise performance in Thoroughbred racehorses. In: EQUINE NUTRITION AND PHYSIOLOGY SOCIETY SYMPOSIUM, 13, 1993, University of Florida. **Proceedings**…p.125-129.

PAGAN, J.D.; GEOR, R.J.; HARRIS, P.A.; HOEKSTRA, K.; GARDNER, S.; HUDSON, C.; PRINCE, A. Effects of fat adaptation on glucose kinetics and substrate oxidation during low intensity exercise. **Equine Veterinary Journal**, London, v.34, p. 33-38, 2002. Supplement.

PALMGREN KARLSSON, C.; JANSSON, A.; ESSÉN-GUSTAVSSON, B.; LINDBERG, J.E. Effect of molassed sugar beet pulp on nutrient utilisation and metabolic parameters during exercise. **Equine Veterinary Journal**, London, v. 34: 44–49, 2002. DOI: 10.1111/j.2042-3306.2002.tb05390.x

POTTER, G.D.; ARNOLD, F.F.;HOUSEHOLDER, D.D.; HANSEN, D.H.; BROWN, K.M. Digestion of starch in the small or large intestine of the equine. **Pferdeheilkunde**, Sonderheft, p.107-111, 1992.

PRATT, S.E.; LAWRENCE, L.M.; WARREN, L.K.; POWELL, D.M. The effect of exercise on the clearance of infused acetate in the horse. **Journal of Equine Veterinary Science**, Wildomar, v.25, p.266-271, 2005.

PRATT, S.E.; GEOR, R.J.; SPRIET, L.L.; MCCUTCHEON, L.J. Time course of insulin sensitivity and skeletal muscle glycogen synthase activity after a single bout of exercise in horses. **Journal of Applied Physiology**, Bethesda, v.103, 1063-1069, 2007.

RIBEIRO, W.P.; VALBERG, S.J.; PAGAN, J.D.; ESSEN-GUSTAVSSON, B. The effect of varying dietary starch and fat content on serum creatine kinase activity and substrate availability in equine polysaccharide storage myopathy. **Journal of Veterinary Internal Medicine**, Lawrence, v.18, p.887–894, 2004.

ROSE, R.J.; HODGSON, D.R.; BAYLY, W.M.; GOLLNICK, P.D. Kinetics of $\dot{V}O_2$ and $\dot{V}CO_2$ in the horse and comparison of five methods for determination of maximum oxygen uptake. **Equine Veterinary Journal**, London, v.22, p.39–42, 1990. DOI: 10.1111/j.2042-3306.1990.tb04732.x

SCOTT, B.D.; POTTER, G.D.; GREENE, L.W.; HARGIS, P.S.; ANDERSON, J.G. Efficacy of fat-supplemented diet on muscle glycogen concentrations in exercising thoroughbred horses maintained in varying body conditions. **Journal of Equine Veterinary Science**, Wildomar, v.12, p. 109-113, 1992.

SIMMONS, H.A.; FORD, E.J. Gluconeogenesis from propionate produced in the colon of the horse. **The British Veterinary Journal**, London, v.147, p.340-345, 1991.

SLOET VAN OLDRUITENBORGH-OOSTERBAAN, M.M.; ANNEE, M.P.; VERDEGAAL, E.J.; LEMMENS, A.G.; BEYNEN, A.C. Exercise- and metabolism-associated blood variables in Standardbreds fed either a low- or a high-fat diet. **Equine Veterinary Journal**, v.34, p. 29-32, 2002. Supplement.

SNOW, D.H.; BAXTER, P.; ROSE, R.J. Muscle fibre composition and glycogen depletion in horses competing in an endurance ride. **Veterinary Record**, London, v.108, p.374-378, 1981. Available at: doi:10.1136/vr.108.17.374

SNOW, D.H.; KERR, M.G.; NIMMO, M.A.; ABBOTT, E.M. Alterations in blood, sweat, urine, and muscle composition during prolonged exercise in the horse. **Veterinary Record**, London, v.110, p. 377-384, 1982.

SNOW, D.H.; HARRIS, R.C.; HARMAN, J.C.; MARLIN, D.J. Glycogen repletion following different diets. In: GILLESPIE. J. R.; ROBINSON, N. E. **Equine exercise physiology 2**. Davis: ICEEP publications, 1987. p. 701-710.

SNOW, D.H.; HARRIS, R.C. Effects of daily exercise on muscle glycogen in the Thoroughbred racehorse. In: PERSSON S.G.B.; LINDHOLM A.; JEFFCOTT L.B.

(Ed.).**Equine exercise physiology 3.** Davis: ICEEP Publications, 1991. p. 299-304.

SNOW, D.H; VALBERG, S.J. Muscle Anatomy, physiology and adaptations to exercise and training. In: ROSE, RJ e HODGSON, DH (Ed.). **The Athletic horse**: principles and practice of equine sports medicine. Philadelphia: Saunders, 1994. chap. 8, p. 162-166.

SYMONS, D.J.; JACOBS, I. High-intensity exercise performance is not impaired by low intramuscular glycogen. **Medicine & Science in Sports & Exercise**, Hagerstown, v. 21, p. 550-557, 1989.

TAYLOR, L. E.; FERRANTE, P. L.; KRONFELD, D. S.; MEACHAM, T. N. Acid-base variables during incremental exercise in sprint-trained horses fed a high-fat diet. **Journal of animal science**, Champaign, v.*73*, n.7, p.2009-2018, 1995.

TINKER, M.K.; WHITE, N.A.; LESSARD, P.; THATCHER, C.D.; PELZER, K.D.; DAVIS, B.; CARMEL, D.K. Prospective study of equine colic risk factors. **Equine veterinary Journal,** London, v.29, 454-458, 1997.

TOPLIFF, D.R.; POTTER, G.D.; DUTSON, T.R. Diet manipulation and muscle glycogen in the equine. In: EQUINE NUTRITION AND PHYSIOLOGY SYMPOSIUM, 8., 1983, Savoy. **Proceedings**…Savoy: Equine Nutrition and Physiology Society, 1983. p. 119-124.

TOPLIFF, D.R.; POTTER, G.D.; KREIDER, J.L.; DUTSON, T.R.; JESSUP, G.T. Diet manipulation, muscle glycogen metabolism and anaerobic work performance in the equine. In: EQUINE NUTRITION AND PHYSIOLOGY SYMPOSIUM*,* 9., 1985, The Kellogg Centrep. **Proceedings**…p.224-229.

TREIBER, K.H.; HESS, T.M.; KRONFELD,D.S.; BOSTON, R.C.;GEOR, R.J.;FREIRE,M.; SILVA, A.M.;HARRIS, P.A. Dietary energy source affects glucose kinetics in trained Arabian geldings at rest and during endurance exercise. **The Journal of Nutrition**, Philadelphia, v.138, p. 964-970, 2008.

VALBERG, S. Glycogen depletion patterns in the muscle of Standardbred Trotters after exercise of varying intensities and durations. **Equine Veterinary Journal**, London, v.18, p. 479–484, 1986. DOI: 10.1111/j.2042-3306.1986.tb03697.x

VERVUERT, I.; VOIGT, K.; HOLLANDS, T.; CUDDEFORD, D.; COENEN, M. Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. **The Veterinary Journal**, 182(1), 67-72, 2009.

VONDEROHE, C. E.; VALASEK, S. I.; MCKIM, B. R.; CAVINDER, C. A.; FLUCKEY, J.D.; SIGLER,D. H.. Effect of dietary starch level on muscle glycogen replenishment in performance horses. **American Society of Animal Science Southern Section Abstract Book**, p. 18-19, 2014.

WALLER A. P.;LINDINGER M. I. Nutritional aspects of post exercise skeletal muscle glycogen synthesis in horses: A comparative review. **Equine Veterinary Journal,** London, v. 42**,** p. 274-281, 2010. DOI: 10.2746/042516409X479603

WILLIAMS, C.S., D.S. KRONFELD, W.B. STANIAR, P.A. HARRIS. Plasma glucose and insulin responses in Thoroughbred horses fed a meal high in starch and sugar or fat and fiber. **Journal of Animal Science**. v.79, p. 2196-201, 2001.