Summer 2016

Protein And Exercise Effects On The Musculature Of The Back In Horses

Taryn Elizabeth Smith

PROTEIN AND EXERCISE EFFECTS ON MUSCULATURE OF THE BACK IN HORSES

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Natural and Applied Science

By

Taryn Smith

July 2016

PROTEIN AND EXERCISE EFFECTS ON MUSCULATURE OF THE BACK IN HORSES

Agriculture

Missouri State University, July 2016

Master of Natural and Applied Science

Taryn Smith

ABSTRACT

This study was designed to examine the effects of exercise on the musculature of the back of ten horses which are used to teach beginning through advanced horsemanship. This was executed by implementing a strict exercise protocol, examining body composition changes and subjecting them to a standard exercise test at the beginning and end of the experimental period in order to determine change in aerobic conditioning. Horses were randomly assigned to one of two treatment groups for six weeks; Purina SuperSport™ (40% CP) and MFA Easy Keeper™ (32% CP). Body weight (BW), body condition score (BCS), rump fat, topline evaluation score (TES), belly circumference, gaskin circumference, and area of the back at the withers, back, and loin were examined during the study. Significant treatment difference ($P<0.05$) for change in BCS and rump fat and a trend for a treatment difference in BW change did occur. All other body parameters showed no diet affect. Variables measured during the SETs and recovery included heart rate (HR), respirations rate (RR), and rectal temperature (RT) as well as arena ambient temperature and wet bulb globe temperature. Statistical analysis was done with the MiniTab® GLM procedure. No significant treatment effects on HR during the SET, end of SET, and 1, 5, 10, and 15 minutes post-SET; RT at beginning and end of SET were found. Significant horse effects on RR at 1, 5, 10, and 15 minute recovery ($P<0.05$) were found. Protein supplementation and exercise did not have any effect on the musculature of the back in horses.

KEYWORDS: horse, protein, exercise, topline, musculature

This abstract is approved as to form and content

____
Gary W. Webb, PhD
Chairperson, Advisory Committee
Missouri State University

PROTEIN AND EXERCISE EFFECTS ON MUSCULATURE

OF THE BACK IN HORSES

By

Taryn Smith

A Masters Thesis
Submitted to the Graduate College
Of Missouri State University
In Partial Fulfillment of the Requirements
For the Degree of Natural and Applied Science

August 2016

Approved:

____
Gary Webb, PhD: Professor

____
Michael Goerndt, PhD: Associate Professor

____
James Hutter, PhD: Associate Professor

____
Julie Masterson, PhD: Dean, Graduate College

ACKNOWLEDGEMENTS

My time here at Missouri State University has been both challenging and enjoyable giving me a chance to grow intellectually and personally. I am thankful for the opportunity to achieve a master's degree. This would not have been possible without the help and support of many people.

I would like to thank Dr. Gary Webb for granting me a position as one of his graduate students. Dr. Webb has a wealth of knowledge and has helped me tremendously through his patience and advice. I would also like to thank Sue Webb for her help and support during my time here. Sue was always willing to help in any way she could during the research as well as in other areas of my graduate work. I am grateful to Dr. Webb and Sue for their support and guidance.

Next, I would like to thank Dr. Michael Goerndt. I admire Dr. Goerndt's attention to detail, and statistical knowledge. If it weren't for him, finding the area of the back in the horses would have been a time consuming and monotonous process. I would also like to thank Dr. James Hutter. He has been a tremendous help in becoming a teacher and receiving my master's.

I would also like to thank my fellow graduate students and student workers both past and present; I would have not been able to complete my research without their help and support. Especially Cassie O'Hara, who was with me every day of the project, helping me in every way, and keeping me company during the long days at the barn.

Lastly, I would like to thank my mother. Without her support I would not have had the drive and stamina to get to where I am today.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Justification of the Study

Protein supplementation is a common practice in many areas of animal production, as well as in humans for dietary purposes. Protein can be an expensive part of the diet in horses and it is necessary to understand the effects of protein supplementation in humans and in animals in order to justify the added expense. There are many studies examining the effects of protein supplementation in swine, humans, and horses (Qin et al., 2015; Hansen et al., 2016; Vineyard et al., 2013). In swine production, soybean meal is the primary source of protein with cottonseed meal becoming a more popular source because it has a high amount of protein but a lower cost. Many studies have shown that protein source and breed of swine rather than the amount of protein fed to swine is what tends to affect growth performance (Qin et al., 2015; Liu et al., 2015; Zhou et al., 2015). In regards to humans, protein supplementation is common, especially in athletes and heavy lifters. There is some controversy in the idea that adults who exercise may need more protein than is recommended for the average sedentary human (Lamont, 1991; ACSM, 2000). Studies on both body builders and elite cyclists show little difference in the amount of protein ingested when compared to gain in muscle or repairing of muscle tissue (Spillane and Willoughby, 2016; Hansen et al., 2016). Protein supplementation is a common practice in exercising horses. There are many studies showing that supplementing more protein than the recommended amount in the exercising horse has

little effect on the horse's body composition (Patterson et al., 1985; Graham-Thiers et al., 2000; Graham-Thiers et al., 2003).

Exercising is of high importance in the equine athlete because developing the correct muscle groups allows the horse to perform at its highest level. The back is one of the most important parts of the horse and supports not only the horse, but the rider as well. Studies examining exercise effects on the horse have shown that by implementing an exercise protocol horse's body composition does change as well as their ability to move at the various gaits (Flora et. al, 2007, Walker et al., 2016). Cardiovascular training is one of the more common types of exercising used in the training of horses. Stretching, as well as physical therapy, are ways to increase the strength in horses especially in the horses back (Oliveria et al, 2015; Paulekas and Haussler, 2009). Through exercise and physical therapy horses will be stronger and better able to support themselves and the rider. Multiple research experiments have been done on the effects of protein supplementation in the exercising horse, as well as exercise effects on the musculature of the horse. More research is warranted to determine if protein supplementation and exercise will affect the musculature of horses, particularly the musculature of the back.

Objective & Null Hypothesis

The objective of this study was to examine the effects of 32% and 40% protein supplementation and an exercise regimen on the musculature of the back in horses by measuring body composition and subjecting them to a standard exercise test (SET) where heart rate, respiration rate, and rectal temperature were measured.

Supplementing protein and implementing an exercise regimen will not increase musculature of the back in horses.

LITERATURE REVIEW

Protein Supplementation in Swine

Overfeeding of protein can be is common in finishing pigs. Soybean meal has been the primary protein source in swine diet with cottonseed meal used as an alternative diet because it has high protein content. Qin et al., (2015) used 72 crossbred gilts in order to examine if there was a difference in the crude protein levels on the intracellular free amino acid profile in the longissimus dorsi muscle. The gilts were broken up into four groups with each group assigned to a specific diet which were as follows: soybean meal with 12% crude protein, soybean meal with 14% crude protein, cottonseed meal with 12% crude protein, and cottonseed meal with 14% crude protein. Average daily gain as well as level of consumption of feed was greater in the gilts offered the cottonseed meal diets. Lower crude protein level also affected average daily feed intake. However, gain to feed ratio was not altered by dietary treatments, and no interaction between dietary protein sources and levels for growth performance was observed (Qin et al., 2015). Neither dietary protein sources nor crude protein levels altered carcass characteristics, including carcass weight, dressing percentage, and back fat depth (Qin et al., 2015). Results of phenylalanine, tryptophan, cysteine, and tyrosine concentrations in the muscle being significantly reduced in cottonseed meal diets as well as a small reduction in histidine, threonine, aspartic acid, glutamine, and serine concentrations shows that dietary protein source rather than protein level altered amino acid profile of the longissimus dorsi (Qin et al., 2015).

Nitrogen excretion can be a major problem in swine production. One way to reduce nitrogen excretion without affecting the growth performance of growing and finishing pigs is by feeding a low-protein diet supplemented with essential amino acids. A study using 120 barrow pigs broken into four treatment groups aimed to determine if protein level and cysteamine supplementation had any effect on growth performance and carcass traits. The four different diets consisted of a normal protein diet (14% CP), a normal protein diet (14% CP) with a cysteamine supplementation (700 mg/kg), a low protein diet (10% CP), a low protein diet (10% CP) with a cysteine supplementation (700 mg/kg). Initial and final body weights and feed consumption were recorded to calculate growth performance including average daily gain, average daily feed intake, and feed conversion ratio (Zhou et al., 2015). Dietary protein levels did not affect growth performance and carcass traits but cysteamine supplementation increased average daily gain and lean percentage as well as decreased feed conversion ratio and back fat but had no effect on average daily feed intake, dressing percentage, and loin eye area (Zhou et al., 2015).

Breed affects the utilization of protein in swine as shown by a study using two different breeds, 48 Bama mini-pigs and 48 Landrace pigs. The study broke the pigs up into 4 groups with each breed being fed one of two dietary treatments. The NRC (National Research Council) diet was formulated to meet the nutrient requirements recommended by NRC and had a high protein/energy ratio, whereas the GB (Chinese conventional) diet was formulated per the recommendations of Chinese National Standard for Swine and had a low protein/energy ratio (Liu et al., 2015). The pigs were

grown from piglets through the finishing stage and after each phase eight pigs were picked at random from each treatment to be weighed, bled, and sacrificed to evaluate carcass characteristics and meat quality. Feed intake was also recorded every two weeks to determine average daily gain (ADG), average daily feed intake (ADFI), and the feed intake to body gain ratio (F/G). Carcass composition was assessed by measuring pre-slaughter body weight, carcass weight, carcass length, backfat thickness, and loin-eye area at the 10^{th} rib (Liu et al., 2015). The results of this study showed many variations in growth performance and carcass quality. Specifically, the diet affected the breeds differently. ADG and ADFI of Bama mini-pigs were lower, whereas F/G was higher when compared with Landrace pigs in the same phase and fed the same diet, but growth performance of Bama mini-pigs did not significantly differ between dietary treatments in any of the three phases (Liu et al., 2015). There was a breed x diet interaction with the Landrace pigs that were fed the GB diet having a higher ADG and ADFI than the ones on the NRC diet. Within each breed, pigs fed the NRC diet had considerably higher dressing percentage than those fed the GB diet, but in Landrace pigs, GB diet promoted carcass length and lean percentage, especially during the growing phase. The results of this study may help in reducing feed cost and minimizing the adverse effects of ammonia release to the environment in swine production (Liu et al., 2015). Species that are selected for heavy muscling require more protein than those not selected for heavy muscling which is supported from these studies specifically examined in swine. However, there have been no studies to show that horses selected for heavier muscling will require more protein than those horses that are of lighter muscling.

Protein Supplementation in Humans

Like other mammals, protein is an essential part of the diet in humans. The Recommended Dietary Allowance (RDA) of protein provided by the United States Food and Nutrition Board of the National Academy of Sciences recommends that the average American adult ingest 0.8 grams of protein for each kilogram of body weight per day (Lamont, 2003). There is some controversy in the idea that athletes should consume more than this recommended amount of protein (Lamont, 1991; ACSM, 2000). Protein and amino acids are metabolized during prolonged exercise, but it does not appear that trained individuals use more than do the sedentary ones (Lamont, 2003). It is unclear whether exercise of longer duration increases the acute requirements of protein at the whole-body level or whether a slightly greater amount of protein or a greater number of repeat feedings is necessary to rapidly and fully restore whole-body protein balance after endurance exercise (Moore et al., 2014).

One study was conducted using 21 male participants who were asked to complete a four day/week heavy resistance training program for eight weeks while supplementing with an excess amount of protein and/or carbohydrate. The supplement treatments were a 312 g-day of a carbohydrate supplement or a protein and carbohydrate supplement (Spillane and Willoughby, 2016). Body composition, muscle performance, and markers indicative of muscle protein synthesis and myogenesis were all assessed with no supplement-induced improvements seen (Spillane and Willoughby, 2016). This was most likely due to the “muscle full effect” in which it is suggested that there must be an upper limit of amino acids delivery before muscle cells would no longer use them as a substrate

for muscle protein synthesis, instead diverting them toward oxidation (Atherton et al., 2010).

Another study utilized elite racing cyclists during a one-week training camp. The subjects were grouped based on weight, maximal oxygen consumption, a five minute all –out performance, and training history, then were randomized to consume a carbohydrate (CHO) beverage or a protein-carbohydrate (PRO-CHO) beverage during each training session, and finally divided into a short distance group versus a long distance group (Hansen et al., 2016). All athletes consumed the same recovery beverage after exercise that contained 18 g protein and 69 g carbohydrate. Each subject ingested one bottle (750 ml) for every hour of exercise they performed. The PRO-CHO beverage contained 0.2 g of protein/kg/h (whey protein hydrolysate with a degree of hydrolysis between 23-29%, Arla Foods Ingredients Group P/S, Viby, Denmark) and 1 g of CHO/kg/h (Maxim Energy Drink, Maxim International, Isheoj, Denmark) while the CHO beverage contained 1.2 g of CHO/kg/h (Maxim Energy Drink, Maxim International, Ishoej, Denmark). Three tests were performed, one on day one before training and after training, and again on day six before training. The protocol for the tests consisted of a standardized warm-up prior to a 10-s peak power test followed by a three minute recovery period and a five minute all-out performance test (Hansen et al., 2016). Blood samples were taken in order to analyze markers of muscle damage by examining creatine kinase levels, lactate dehydrogenase, and myoglobin, as well as to examine cortisol levels. Saliva samples were taken to examine immunoglobulin A levels in order to determine immune function.

Results of the study showed that the intake of protein during training did not have a marked influence on the changes in markers of muscle damage, cortisol, or immune function during the training camp (Hansen et al., 2016).There was no interaction between treatments in reference to lactate dehydrogenase and plasma myoglobin was below detection level in both treatments. Creatine kinase, an enzyme found in muscle tissue, was affected by treatment group, being higher in the mornings for the protein-carbohydrate group but having no significant change in the carbohydrate group. Creatine kinase levels increase as muscles are overworked and become damaged. This would suggest that the protein-carbohydrate group were experiencing more muscle damage. Cortisol levels did increase towards the end of the week but there was no significant interaction between treatments. There was no significant difference between treatments in regards to salivary immunoglobulin A levels. Body weight was also examined and the change that did occur was not significantly different between groups (Hansen et al., 2016).

Protein Requirements in Horses

Studying the effects of nutrition on horses can be a difficult task. In severe cases of malnutrition the effects are considerably noticeable, yet most horses used for research in nutrition are already in good condition and the effects can be extremely minor and difficult to measure. Another factor affecting research in nutritional effects on horses is the small number of horses used in most studies. This is most likely due to lack of resources and funding (Hintz, 1994). However, knowing the dietary needs of horses is

important when examining what effects nutritional changes can have. Protein requirements, in specific, are difficult to define in the horse. Protein is a major component of most tissues in the body, second only to water. Protein is made up of amino acids. The level of essential amino acids in protein is the limiting factor on how much protein a horse can process. Due to the essential amino acid content of most feedstuffs fed to horses, lysine is usually the amino acid which limits protein utilization. The challenge in feeding horses is to provide adequate quantities of protein that will allow for sufficient concentrations of circulating amino acids in the blood that the body can draw on to synthesize tissues, enzymes, and hormones, as well as repair tissues (NRC, 2007).

Protein requirements vary based on the physiological state of horses. At maintenance the minimum protein requirement can be calculated using the equation BW x 1.08 g CP/kg BW/d. However, some horses at maintenance are more physically active without forced exercise and should have their protein requirement be based on the equation BW x 1.44 g CP/kg BW/d. For the average horse at maintenance using 1.26 g in the equation is sufficient. During growth protein requirements can be determined by the equation (BW x 1.44 g CP/kg BW) plus ((ADG x 0.20/E)/0.79) which depends on the average daily gain of the horse as well as efficiency of use of dietary protein represented in the equation as E (NRC, 2007). The efficiency of use of dietary protein is based on the horses age and can be estimated as the following: 50 percent for horses 4-6 months of age, 45 percent for horses 7 and 8 months of age, 40 percent for horses 9 and 10 months of age, 35 percent for horses 11 months of age, and 30 percent for horses 12 months of age or older (NRC, 2007). During early pregnancy, pregnant mares have the same protein

requirements as the average horse at maintenance. However, from the fourth month until parturition ((fetal gain in kg/0.5)/.79) is added to the equation. The protein requirement for exercising horses depends purely on the workload. Depending upon exercise protocol, the more a horse works the more muscle maybe gained and the more nitrogen lost through sweat. The equations used for protein recommendation in exercising horses are in addition to what is needed at maintenance. The equation is BW x MG plus ((BW x SL x 7.8 g/kg)/0.50)/0.79). MG is muscle gain and is estimated to be 0.089 g CP/kg BW, 0.177 g CP/kg BW, 0.266 g CP/kg BW, 0.354 g CP/kg BW for light exercise, moderate exercise, heavy exercise, and very heavy exercise while SL is sweat loss and is estimated to be 0.25, 0.50, 1 and 2 percent for light exercise, moderate exercise, heavy exercise, and very heavy exercise (NRC, 2007).

Protein is an expensive component of the diet of all horses, thus the effect of exercise on the protein requirement for optimal growth is of practical significance (Orton et al., 1985). Two experiments were conducted in order to determine the effects of the level of dietary protein and exercise on growth rates of horses. The first experiment was comprised of eight two-year-old horses that were assigned to one of four treatment groups: low protein (6% CP) with exercise, low protein without exercise, high protein (12% CP) with exercise, and high protein without exercise. At the start and end of the 42 day experimental period the following measurements were taken on each horse: height at withers, hear girth circumference, cannon bone length, joint to between the distal and proximal rows of carpel bones, body length, and distance between the tuber ischia and lateral tuberosity of the humerus. Experiment two consisted of eight nine-month old

horses. The horses were assigned to a treatment group described in experiment one, but after 40 days they were kept on the same diet and switched to the other exercise regime. The crude protein levels were higher (8% and 14% respectively) in the second experiment. In both experiments, exercise increased the rate of body weight gain of horses fed the low protein diet and the efficiency with which the apparently digestible crude protein was used for body weight gain for both exercised and non-exercised horses. There were no significant differences in any body parameters measured in any of the experimental periods (Orton et al., 1985). The results of this study show that growing horses can be fed lower protein concentrations if they are exercised. Because feed intake will be increased to meet the energy requirements as the horses are exercised, protein concentration of the rations can be reduced without lowering total protein intake.

Most adult horses are overfed protein due to the fact that most feedstuffs contain more protein than recommended and, combined with the protein found in hay, exceeds the recommended level. There is no evidence that performance can be enhanced by feeding diets containing concentrations of protein greater than the 11% (dry matter basis) as suggested by NRC (Hintz, 1994). When energy intake or energy stores are adequate, exercising horses need only small amounts of protein above maintenance requirements (Meyer). If the protein-to-energy ratio is maintained, the additional feed intake required to supply the necessary energy will also supply the additional protein needed (Hintz, 1994). Some negative effects of increased protein in the diet of horses are the following; water requirements increase and plasma urea level increases causing more urea to enter the alimentary tract thus increasing the risk of intestinal disturbances such as

enterotoxemia (Meyer). Excessive protein intake can increase renal urea excretion which then can cause higher ammonia concentrations in the air of a barn. This can result in stress to the respiratory system which can reduce resistance to infections.

Although many horses are fed diets containing a higher level of protein than required, protein is needed during physical conditioning to support muscle hypertrophy and repair and to replace nitrogen lost in sweat (Graham-Thiers et al., 2003). In one study 18 mature horses were used to study dietary crude protein requirements for maintenance and exercise (Patterson et al., 1985). The treatments consisted of three levels of crude protein at 8.5%, 7.0%, and 5.5% and three levels of physical activity at intense work, medium work, and maintenance. A mare and a gelding were assigned to each treatment resulting in a total of nine treatment groups. Blood samples and body weight were taken every seven days, pulse and respiration rates were taken on day three, 31, and 59 of the medium and intense working horses only, and urine samples were collected from all horses during the second, sixth, and tenth week of the trial. The exercise protocol for the intense and medium work horses consisted of workouts five days a week for 50 minutes. The intensity of the workout is the only thing that varied among the two groups. The arena work for the intense work horses was 10 minutes of walking, 20 minutes of trotting, 15 minutes of cantering, and five minutes of hard-galloping. The arena work for the medium work horses was 20 minutes walking, 20 minutes long-trotting, and 10 minutes cantering (Patterson et al., 1985). Some differences occurred when examining the heart rates, respirations rates, and body weights. In the high protein treatments lower heart rates were maintained throughout post-exercise measurements. Respiration rates did

not differ among dietary treatments; however, fifteen minutes post exercise the low-protein group still had an elevated respiration rate. Body weight decreased over the study period; horses fed the low protein maintained 94.7% of their starting body weight, the medium-protein group maintained 96.0%, and high-protein group 97.5% (Patterson et al., 1985). There were no consistent effects of exercise upon blood or urine characteristics that would suggest supplemental protein is required for exercise beyond maintenance (Patterson et al., 1985).Plasma urea nitrogen concentrations among all treatments decreased, serum globulin and total proteins were not affect by the level of protein in the diet, plasma albumin concentrations were within normal range for all horses and was not affected by the level of protein, and the ratio of albumin to globulin was similar among protein treatments.

A separate study with mature horses used ten Arabian horses assigned to two different diets; the first one consisted of a low protein diet (7.5% CP) that was fortified with 0.5% lysine and 0.3% threonine while the second one consisted of a high protein diet containing 14.5% CP. A nine week conditioning program with an exercise test performed at the end was implemented. Weight, body condition score, blood and urine samples taken every two weeks during the trial were used for analyzing the effects of the diet treatments. Average starting weight of 436±17 kg compared to average ending weight of 445±17 kg as well as body condition scores of 5.2±4 in the beginning and 5.7±4 at the end showed no significant difference between diets (Graham-Thiers et al., 2000). P values of 0.53, 0.93, and 0.29 for plasma albumin, protein, and creatine respectively showed no effect of diet during both the condition period and the exercise

test. Plasma urea-N concentration, urea-N:creatinine ratio, urine urea, uric acid concentrations, urine urea:creatinine and uric acid:creatinine rations were all greater in the high protein diet group versus the low protein diet group. The levels of urine urea in the high protein diet group are reflections of the level of nitrogen in the diets and demonstrate the increased excretion of urea (Graham-Thiers et al., 2000). Overall, the results indicate that the LP diet supplemented with limiting amino acids supported normal protein status during a nine-week conditioning program and a repeated sprint test (Graham-Theirs et al., 2000).

Graham-Thiers et al., (2003) conducted a similar study involving 12 Arabian horses assigned to four different diets; high-protein (14.5% CP), high-fat (13% including 10% added corn oil), a low-protein (7.5% CP), high-fat, a high-protein, low-fat (3%), and a low-protein, low-fat. The horses were then put through a 17 week period of interval training and repeated sprints as well as a standardized exercise test at week 4, 16, and 17. Diet did not affect body weight change with the average starting weight at 419±15 kg and the average ending weight oat 459±15 kg (P=.41). BCS was lower in the low protein, high fat group. Results of the study showed similar results to that of the previous study with plasma urea nitrogen concentrations being higher in the high protein diets. There was no effect of diet on plasma albumin, total phosphorous concentrations, or protein. The results of the study showed no detrimental effect of restricted dietary protein or fat supplementation on the apparent protein status of the horses over the 17 weeks of the study or during the sprint exercise tests (Graham-Thiers et al., 2003).

As mentioned previously many times horses are overfed crude protein. However, because most of the feedstuffs used in equine diets are low in lysine and methionine these diets may be low in those two essential amino acids. Therefore, if a supplement containing higher levels of those amino acids were fed, it might be possible to feed a ration lower in crude protein. This approach is similar to recent efforts to lower total protein in swine diets as discusses in previous sections of this literature review. Research conducted at the Purina Animal Nutrition Center examined the effects of daily administration of the Purina SuperSport Supplement™, an amino acid-based supplement, on aspects of muscle development in exercising horses (Vineyard et al., 2013). The experiment was comprised of 16 horses that were assigned to one of two treatment groups for 56 days, the first group being fed Purina SuperSport Supplement™, at 45.3% CP and the other being fed alfalfa pellets at 17.4% protein. All horses were exercised on a treadmill three to four times a week and were weighed and examined for BCS at day 0, day 28, and day 56. Rump fat thickness, forearm, and gaskin circumference were also assessed. BCS remained the same in both groups, while rump fat and body weight decreased in the Purina SuperSport Supplement™ group. Forearm and gaskin circumference decreased in both groups over time (Vineyard et al., 2013). The results of this study show that daily feeding of an amino acid based supplement to exercising horses may support muscle development.

Exercise Effects on the Musculature of the Back in Horses

Exercising is essential to the health of the equine athlete. Developing the correct muscle groups so that a horse is able to perform at its highest level is a goal for most equestrians. The back, being one of the most important parts of the horse, facilitates movement, allows for major extension at the various gaits, requires flexibility through lateral bending, and supports the rider. There has been limited investigation on the effect of development of the muscle groups responsible for movement and stability of the neck and back, and the consequential effect on back kinematics (Walker et al., 2016). Body condition scoring is used most often when determining the overall health of the horse but evaluating muscle development has been a difficult task to create a scale for.

In this study a muscle score scale was created to examine how it related to back kinematics Horses used in the study, all having been trained in dressage style riding, were assessed by a veterinarian before exercising and manual palpation of the left and right sides of the neck, abdomen, thoracic region, lumbosacral region, pelvis, and hind limbs and a muscle score of 1 to 5, with 5 being the highest degree of muscle development, was assigned to each horse based on visual assessment (Walker et al., 2016). The horses were then ridden at a collected sitting trot while being filmed using a high-speed video motion camera on both sides of the horses.

The results of this study showed that muscle score did have an effect on kinematics in the dressage horse. Smaller lumbosacral angle was associated with greater muscle score and with the thoracic muscle development during the suspension phase which limited passive flexion of the back at the end of the stance phase and facilitated

hind limb propulsion during the swing phase (Walker et al., 2016). The researchers admitted that the muscle score they used has many limitations and also suggested use of a flexible ruler to help measure symmetry.

It is clear that implementing an exercise program does have positive effects on the horse. The equine skeletal muscle has considerable potential to adapt during training and these adaptations have important physiological implications that influence power maximum velocity of shortening (speed), generation (strength), and resistance to fatigue (stamina) (Rivero, 2007). There are many reasons to condition horses; reduce exercise-induced injuries, enhance the health of the horse, less stress during physical activity, decreased time to recover, and less fatigue after exercise (Hintz,1994). However, there are very few experiments that focus on the effects of various training methods on muscle gain in the horse.

It can be very difficult to determine the strength of the horses back. Body condition scoring is frequently used as a guide for nutritional advice but there is no comparable scale for evaluation of muscle development (Walker et. al, 2016).When examining muscle conditioning in the horse; there are various regions on the horse that can be evaluated. In one study the longissimus dorsi muscle was chosen for ultrasound measurements because it was easy to access and provided good ultrasonographic images. The longissimus dorsi is highly worked during exercise because it is responsible for the extension and flexion of the vertebral column (Flora et. al, 2007). At maximum extension, it is responsible for maintenance of posture, elevation and support of the head, and supporting the weight of the rider.

In one study twelve Arabian horses were divided into two groups. The first group was fed 75 g creatine monohydrate a day while the control group was fed no supplementation. Each horse was evaluated monthly by examining body weight and body condition score. During the first 30 day aerobic training period the horses were worked three times a week on alternating days on a treadmill completing 10 km in a time of 50 minutes. The second 30 days training was increased to 15 km in a time of 60 minutes and the third 30 days was increased to 20 km in a time of 80 minutes. On top of the consistent pace of the aerobic training sessions, a speed type of training was implemented once a week, involving sudden, rapid, relatively short bursts of speed interspersed throughout the exercise (Flora et. al, 2007).

The main finding of this study was the lack of a significant effect of creatine supplementation on longissimus dorsi muscle in response to endurance training (Flora et. al, 2007). Neither body weight nor body condition score differed significantly between groups. There was an increase in the cross-sectional area of the longissimus dorsi muscle during the course of training, thus improving body composition of the horses by increasing fat-free mass. It was in the first month of training that the cross-sectional area of the longissimus dorsi muscle increased the most and the thickness of the layer of fat diminished. (Flora et. al 2007) By implementing an exercise protocol for the horses in this study, muscling increased and fat layer decreased, with creatine supplementation showing no effect over the control group.

Therapeutic Exercise Effects on the Musculature of the Back in Horses

As previously discussed, implementing an exercise program for horses is essential to their health and well-being, allowing them to perform at a desired level. In most conditioning studies the focus has been on cardiovascular fitness in specific sports (Oliveria et al, 2015). Strength training can be a major benefit to horses as shown in one study that aimed to determine the effects of gymnastic training (GYM) and dynamic mobilization exercises (DMEs) on therapy horses. GYM utilizes stretching and strengthening exercises in order to prevent injury and to aid in rehabilitation from injuries. DMEs target the horse's cervical and thoracolumbar intervertebral joints through voluntary movements that move and stabilize the joints (Oliveria et al, 2015).

The study was conducted for three months. All horses were regularly used in hippotherapy sessions. The horses were assigned to three groups; sedentary (SED), DMEs, and DMEs plus GYM. The DME exercise routine consisted of three cervical flexion exercises (chin to chest, chin between carpi, and chin to fore fetlocks), a cervical extension exercise, and three lateral cervical bending exercises performed to the right and left sides (chin to shoulder, chin to flank, and chin to hind fetlock) while the GYM group performed DMEs as described together with exercises to recruit and strengthen the abdominal muscles and pelvic-stabilizing muscles (pelvic tilting, backing up, walking around tight turns, and stepping over obstacles at walk) (Oliveria et al, 2015). To assess the effects of these exercises on the horses they took videos of the horses stride length and tracking distance and took ultrasonographic measurements of the thickness of the longissimus dorsi and the cross-sectional area of m. multifidi (MM). The results of this

study showed that there was an increase in stride length and tracking distance in the GYM group. Stride length was significantly longer in the GYM group compared to the SED or DME groups. Tracking distance was negative in all groups initially, but increased significantly only in the GYM group. The longissimus dorsi muscle did not change in any group; however, the cross-sectional area of multifidi (MM) did increase in both the DMEs and GYM groups (Oliveria et al, 2015).

Physical therapy is becoming a common practice to help maintain and improve the health of the horse. Horses must cope with the demands of carrying a rider in addition to their own body weight. Many riders believe that the horse is naturally adept at supporting the rider's weight, when in reality ill-fitting and improperly used equipment, novice riders, and heavy riders often interfere with the horse's self-carriage, balance, and movement, potentially causing or contributing to pathologic conditions (Paulekas and Haussler, 2009). Spinal mobility is important, allowing the horse to support its own weight, carry a rider, and maintain fluid and elastic movement during locomotion or athletic events. As previously discussed, the longissimus dorsi muscle is extremely important in the horse to maintain posture, allow for extension, and support the rider. It is important that this muscle is relaxed and free to contract to allow and control dorsoventral and lateral movements of the spine in sequence with the gait cycle. If the longissimus dorsi is in a constant state of contraction or chronic hypertonicity and fails to or is unable to relax, the horse will be unable to elevate its back, produce lateral movements of the spine, or readily support the added weight of the rider (Paulekas and Haussler, 2009).

Touch therapy is used as a newer form of physical therapy. Linda Tellington-Jones has developed and promoted this type of touch therapy in a collection of techniques named the Tellington Touch Equine Awareness Method, or Tellington TTouch. It is considered to improve behavior, performance, and well-being of horses and enhance the relationship between horse and rider, but no controlled studies exist to support these claims (Haussler, 2009). One type of Tellington TTouch method is placing a Thera-band in a figure-8 configuration around the horse's trunk to produce a rhythmic, sensory stimulus that is timed with the gait cycle with the goal being to coordinate and synchronize the activity of the hindquarters with the forehand and to facilitate protraction of the pelvic limbs (Paulekas and Haussler, 2009). Through physical therapy and touch therapy along with exercising, back pain in horses can be managed and improved to allow the horse to perform at the optimal level.

MATERIALS AND METHODS

Diets and Treatments

In order to determine if a combination of exercise and protein supplementation would result in an improvement in the back musculature of adult horses a six week study was conducted. Ten Quarter horses (8 geldings and 2 mares), ranging in age from 3 – 19 years old and weighing 506 to 637 kg (mean 572 kg), with all having poor back conformation or lack of musculature in the loin region, were chosen to complete a six week exercise regimen. At the beginning of the study the horses were randomly assigned by housing (drylot, pasture or stall) to one of two protein supplement treatment groups. Treatment one horses were fed MFA Easy Keeper™ (MFA INC, Columbia, MO) containing 32% crude protein while treatment two horses were fed Purina SuperSport™ (Purina Animal Nutrition, Gray Summit, MO) containing 40% crude protein. To determine the horses' fitness level and any improvement in conditioning aerobic standard exercise test (SET) was performed at the beginning and end of the study. The horses were broken up into two exercise groups, A and B, with six horses exercising Monday, Wednesday, Friday (Table 1) and four horses exercising Tuesday, Thursday, and Saturday (Table 2). All the horses had been previously used in the Missouri State University Equestrian Program and had the skill needed to complete the six week study.

All horses had free access to water at all times. All horses were either had free access to pasture, a round bale of mixed grass hay or were offered 9 kg (as fed basis) of the mixed grass hay (Table 3) such that hay intake range from 1.3 to 1.5% of BW on a dry matter basis. Based on a crude protein content of 8% for this hay horses were

consuming a minimum of 650 grams of crude protein from the hay. In addition horses were fed from 2-4 kg of MFA Easykeeper 14% Performance Ration™ (MFA INC, Ash Grove, MO). This would result in a crude protein in take of 280 to 480 grams per day when added to the hay the crude protein intake would be 790-1,130 grams from those two sources (Appendix Table A). This compares to requirements which range from 734 to 981 grams of crude protein for horses of these weights doing moderate work (NRC, 2007). Horses' body weights were taken at the end of every week during the study and the amount of 14% concentrate fed was adjusted in order to maintain body weight (Table 3). Horse four was originally fed two kg of MFA grain a day as well as the protein supplement. This horse's weight fluctuated therefore grain intake was increased to four kg of MFA grain a day. The feeding schedule was set this way in order to mimic the previous study of Vineyard et. al. (2013). Also, in order to mimic the same protocol followed by Vineyard et. al, (2013), horses were fed an amount of supplement to provide 0.2 grams of crude protein per kilogram of body weight per day. Because Purina SuperSport™ contains 40% crude protein as compared to MFA Easy Keeper™ which contains 32% crude protein, it was necessary to feed more actual supplement to the MFA treatment group in order to provide the same amount of crude protein. Using NRC values for the lysine content of the hay or pasture and the published lysine content of the supplements (NRC, 2007) all horses consumed adequate lysine to meet or exceed the requirements for this amino acid (Appendix table A). Body condition score (BCS) (Table 4), Topline Evaluation Score (TES) (Table 5), Rump Fat, Gaskin circumference, and Belly size were measured at the beginning (Table 6 and Table 7), four weeks, and end of

the six week study. All procedures involving the use, care, and management of the horses were approved by the Institutional Animal Care and Use Committee (IACUC) of Missouri State University, (IACUC # 15.028.0-A).

Topline Evaluation Scoring (TES)

The Topline Evaluation Scoring (TES) is a system developed by Progressive Nutrition (Hopkins, MN) to evaluate muscle development in the horse. The system examines the back, loin, and croup because these spots are easily identified and good indicators of the muscle status of the horse. This method of evaluation is hands on and visual. It is a scale using letters instead of numbers starting with the letter A and ending with D. A is ideal muscling over all while D indicates muscle atrophy in the three areas. A description of each grade is detailed in Table 5. For statistical analysis purposes numbers of one through four were used. There have been no controlled studies to validate this system or confirm that it is a true measurement of back strength or muscling.

Exercise Protocol

Exercise protocol one consisted of two poles placed 15 m apart from the center of each pole. The horses were fitted with a TellingTTouch (The Ultimate Horse Training Book, 2006) body wrap consisting of resistance bands that were wrapped around their chest and rump and conjoined just behind their withers (Paulekas and Haussler, 2009). The horses were lunged on a 6.4 m cotton line over the poles, moving directly over the center of the two poles (Figure 1). The horses trotted nine laps to the left then reversed

directions and trotted nine laps to the right. This was executed three times. The horses then loped nine laps to the left and transitioned down to a trot and executed nine more laps, changed directions and repeated that. Direction was changed again and nine more laps were executed at a trot. Direction changed a final time and nine more laps were executed (Table 8). The horses did this exercise twice a week alternating the start direction each time so as to even out the direction the horses bent.

Exercise two consisted of one pole placed along the rail at a certain height (Figure 2). The heights were 35 cm, 46 cm, and 56 cm and the horses were assigned a height based on their jumping abilities. Horses were lunged on a 6.4 m cotton line at a trot for nine laps in both directions. Horses were then asked to jump the pole nine times in both direction and then were brought back down to a trot for nine laps each direction (Table 9). Horses did this exercise once a week alternating the start direction.

Standardized Exercise Test (SET)

At the beginning and end of the study all horses were subjected to a standardized exercise test (SET) which was an aerobic test designed to measure the horses' fitness and determine change in condition. The first SET tests and recovery periods were conducted inside an indoor arena over two consecutive days from 1300 to 1700 hours. The second SET tests and recovery periods were conducted inside the same arena over two consecutive days from 1000 to 1500 hours. Temperature and humidity measurements were taken using a Wet Bulb Globe Thermometer (Extech Instruments Corporation, Waltham, MA) at the beginning and end of each SET. The SET was designed to have the

horse's heart rate raised to a range for the specific gait (aerobic ≤ 150 beats per minute). The desired range for the aerobic SET was at a walk 50-70 beats per minute; the trot 71-110 beats per minute; and the lope 111-150 beats per minute. The speed of travel for each horse was manipulated to keep the heart rate in the specified range.

Three riders rode the same horses for both SETs. Two horses had a different rider during the second SET compared to the first SET but the riders were of similar body build and weight. The same riders rode the same horses for both SETs in order to help standardize rider/tack weight. In this project, horses performed the SET in pairs so as to help standardized speed. Speed was measured by setting two cones 6.1 meters from the long side of the arena rails, 7.62 meters from the short side of the arena rails, and 18.3 meters between the two cones. Horses were timed for the amount of time it took to travel 18.3 meters at both the trot and lope in both directions with the use of a hand held timer and the time was recorded in seconds.

The aerobic SET averaged 25 minutes, and consisted of three series which included walk, trot, and lope with change in directions at each gait that was either a stop or rollback, or a simple change in direction (Table 10). The first series served as a warm up, it consisted of a walk 1.0 minute each way, a trot 1.5 minutes each way, and a lope 1.5 minutes each way. The second series included a walk 0.5 minutes each way, a trot 2.0 minutes for each way, and a lope 2.0 minutes each way. The third series consisted of a walk 0.5 minutes each way, a trot 1.5 minutes each, and a lope 2.0 minutes each way. The total minutes for each gait are four minutes at a walk, ten minutes at a trot, and eleven minutes at a lope.

In association with the SET, variables measured included respiration rate, heart rate, rectal temperature. Respiration rates were measured at rest, end of SET, and 1, 5, 10, and 15 minutes after the SET. Respiration rates were measured by counting how many breaths the horse took in 15 seconds and multiplying that total by 4. Rectal temperatures were taken with a digital thermometer (Walgreens Company, Deerfield, IL) at rest and at the end of recovery.

Target heart rate ranges were used to standardize workload so each horse was fitted with an onboard heart rate monitor (Polar Equine, Fleurier, Switzerland). The heart rate monitors were also used to measure heart rates at rest, during the SET, end of SET, and 1, 5, 10, and 15 minutes post-SET. Speeds were manipulated to keep the horse's heart rate in a specific range (walk: 50-70 bpm, trot: 71-110 bpm, and lope: 111-150 bpm). Speed was not used to standardize workload because of horse stride variability. This protocol was adapted from a previous study (Webb et al., 2012).

Back Area

Total back area at three positions (withers, back, loin) were measured using a flexible ruler (GoldStar Tools, Los Angeles, CA) shaping it to the position on the horse, and tracing the shape of the ruler onto a large sheet of paper. This was done at the beginning, four weeks, and end of the six week study as well. These drawings were then transferred to graph paper, digitalized, and ran through the program ArcGIS 10.2.2 ESRI to determine the total area, as well as the area of the left side and right side of the graphs.

Statistical Analysis

In order to determine if horses gained aerobic condition during the experimental period the heart rates during and following the SETs and respiration rates and rectal temperatures during recovery were compared by analysis of variance for differences in SETs and treatments. The general linear models procedure in MiniTab® (17^{th} edition, State College, PA) was used. Treatment and SET were set as fixed effects to compare differences in heart rate, respiration rate and rectal temperatures during and following the SETs. Temperature and humidity during the test, % of the horse's weight carried and speed at the trot and lope were used as covariates. Inferences were made based on a type-I error rate of 0.05. This is the same method of analysis used to analyze the results of the previous experiment from which this SET was taken (Webb et. al, 2012) This same procedure was used to compare differences in back area between supplement treatment groups. For this analysis supplement treatment and horse were considered fixed effects. Because the values entered were differences between the beginning and end of the experiment there was one value for each horse for each position therefore only a total n of 10, resulting in 9 degrees of freedom.

Table 1. Exercise schedule for exercise group A horses used for six weeks.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1						
Workout 1 Start to the Left	Rest	Workout 2 Start to the Left	Rest	Workout 1 Start to the Right	Rest	Rest
Week 2						
Workout 1 Start to the Left	Rest	Workout 2 Start to the Right	Rest	Workout 1 Start to the Right	Rest	Rest

Table 2. Exercise schedule for exercise group B horses for six weeks.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1						
Rest	Workout 1 Start to the Left	Rest	Workout 2 Start to the Left	Rest	Workout 1 Start to the Right	Rest
Week 2						
Rest	Workout 1 Start to the Left	Rest	Workout 2 Start to the Right	Rest	Workout 1 Start to the Right	Rest

Table 3. Body weight during the study at three separate times, housing, hay/pasture, grain intake based on change in body weight, and protein supplementation by horse.

Horse	Treatment	Body Weight (kg) 6/9/15	Body Weight (kg) 7/4/15	Body Weight (kg) 7/22/15	Housing	Forage*	Grain (kg) Period 1	Grain (kg) Period 2	Supplement (g)
1	MFA(1)	639	614	614	Pasture	*ad libitum*	2	2	390
2	MFA(1)	634	593	641	Dry Lot	Hay *ad libitum*	4	5	400
3	MFA(1)	616	584	589	Stall	9 kg Hay	3	3	370
4	MFA(1)	555	532	539	Stall	9 kg Hay	2	4	335
5	MFA(1)	507	493	468	Pasture	*ad libitum*	2	2	310
6	Purina(2)	618	614	616	Pasture	*ad libitum*	2	2	310
7	Purina(2)	564	523	566	Dry Lot	Hay *ad libitum*	4	4	280
8	Purina(2)	536	532	548	Pasture	*ad libitum*	2	2	270
9	Purina(2)	532	530	525	Stall	9 kg Hay	3	3	255
10	Purina(2)	525	521	518	Stall	9 kg Hay	4	4	260

*All forages were Fescue, Orchard Grass, and Clover mix

Table 4. Body condition scoring chart adapted from Henneke et al. (1983).

Condition	Score	Description
Poor	1	No fat cover, severe emaciation, ribs, tail head, spinous processes are projecting prominently, structure of the withers, shoulders and neck and noticeable
Very Thin	2	Emaciation, slight fat covering over spinous processes, bone structure is still noticeable
Thin	3	Spinous processes cannot be felt, slight buildup of fat over ribs, tail head is prominent, withers, shoulders, and neck are thin
Moderately Thin	4	Faint outline of the ribs, fat can be felt around the tail head, withers, shoulders, and neck not obviously thin
Moderate	5	Back is level, ribs can be easily felt but are not visible, withers appear rounded, neck and shoulder blend in with the body
Moderately Fleshy	6	May have a slight crease down the body, fat over the ribs feels spongy and tail head fat feels soft, fat is being deposited along the withers and neck and behind the shoulders
Fleshy	7	Crease down the back is becoming more noticeable, individual ribs can be felt but there is fat in-between them, fat is deposited along the neck and wither and behind the shoulders
Fat	8	Crease down the back, difficult to feel ribs, thickening of the neck, area along the wither and behind shoulders are filled with fat, fat accumulation over the rump
Extremely Fat	9	Obvious crease down the back, bulging fat around the tail head, shoulders, neck , and withers, patches of fat over the ribs

Table 5. Topline Evaluation Score (TES) as designed by Progressive Nutrition to assess current muscle status in horses.

GRADE	VISUAL DESCRIPTION
A – Ideal	• This horse has ideal muscle development for its body type. • Muscle is full beside the withers and along the spinal column, such that the vertebrae cannot be seen. • The hip is full and the stifle muscles are defined. • This horse should be able to perform work that requires use of these muscle areas.
B - Sunken beside the withers and back	• This horse is adequately muscled, except it is sunken beside the withers and back. • You may have trouble fitting this horse with a saddle, so the horse may develop soreness. • This can negatively impact attitude and performance.
C - Sunken from the withers through the loins	• This horse is sunken from the withers through the loins. • Muscles beside the withers remain sunken-in on either side. • Back and loin areas appear boney. • Vertebrae will be higher than the muscles beside them. • Muscling over the hip and hindquarters is adequate. • These underdeveloped muscles in the back and loin area may become sore and performance using the back will be difficult
D - Entire topline and hip are poor	• The entire topline and hip are affected. • The hip appears pointed at the top since the vertebrae are higher than the muscles. • The muscles appear very flat over the croup. • In the most severely affected horses, the stifle area is also narrowed. • This horse will lack the strength and stamina to sustain performance.

http://www.prognutrition.com/pn/nutrition-information/top-line-evaluation-system/index.jsp

Table 6. Horses' exercise group, age, sex, initial weight, initial body condition score (BCS), and initial topline evaluation score (TES), and supplement amount assigned at beginning of study for group one fed MFA Easy Keeper™.

Horse	1	2	3	4	5
Exercise Group	A	A	B	B	A
Age	19	12	8	3	3
Sex	G	G	M	G	G
Weight (kg)	639	634	616	555	507
BCS	7	5	7	6.5	6
TES	A	C	B	A	C
Supplement (g)	390	400	370	335	310

Table 7. Horses' exercise group, age, sex, initial weight, initial body condition score (BCS), and initial topline evaluation score (TES), and supplement amount assigned at beginning of study for group two fed Purina SuperSport™.

Horse	6	7	8	9	10
Exercise Group	A	A	A	B	B
Age	16	15	4	16	16
Sex	G	G	G	M	G
Weight (kg)	618	564	536	532	525
BCS	5.5	5	6	5.5	6.5
TES	C	D	C	B	C
Supplement (g)	310	280	270	255	260

Table 8. Exercise protocol one for all horses used twice a week for six weeks.

Direction	Speed	Number of Laps
Left	Trot	9
Right	Trot	9
Left	Trot	9
Right	Trot	9
Left	Trot	9
Right	Trot	9
Left	Lope	9
Left	Trot	9
Right	Lope	9
Right	Trot	9
Left	Trot	9
Right	Trot	9

Table 9. Exercise protocol two for all horses used once a week for six weeks.

Direction	Speed	Number of Laps
Left	Trot	9
Right	Trot	9
Left	Jump	9
Right	Jump	9
Left	Trot	9
Right	Trot	9

Table 10. Standardized Exercise Test (SET) for all horses at the beginning and end of the study.

Gait	Time (min) at gait	Time into Test	Direction Change
Walk	1	1	Change Direction
Walk	1	2	
Trot	1.5	3.5	Stop Roll Back
Trot	1.5	5	Stop Roll Back
Lope	1.5	6.5	Change Direction
Lope	1.5	8	
Walk	0.5	8.5	Change Direction
Walk	0.5	9	
Trot	2	11	Stop Roll Back
Trot	2	13	Stop Roll Back
Lope	2	15	Change Direction
Lope	2	17	
Walk	0.5	17.5	Change Direction
Walk	0.5	18	
Trot	1.5	19.5	Stop Roll Back
Trot	1.5	21	Stop Roll Back
Lope	2	23	Change Direction
Lope	2	25	Stop/End of Test

Figure 1. Diagram of exercise protocol one.

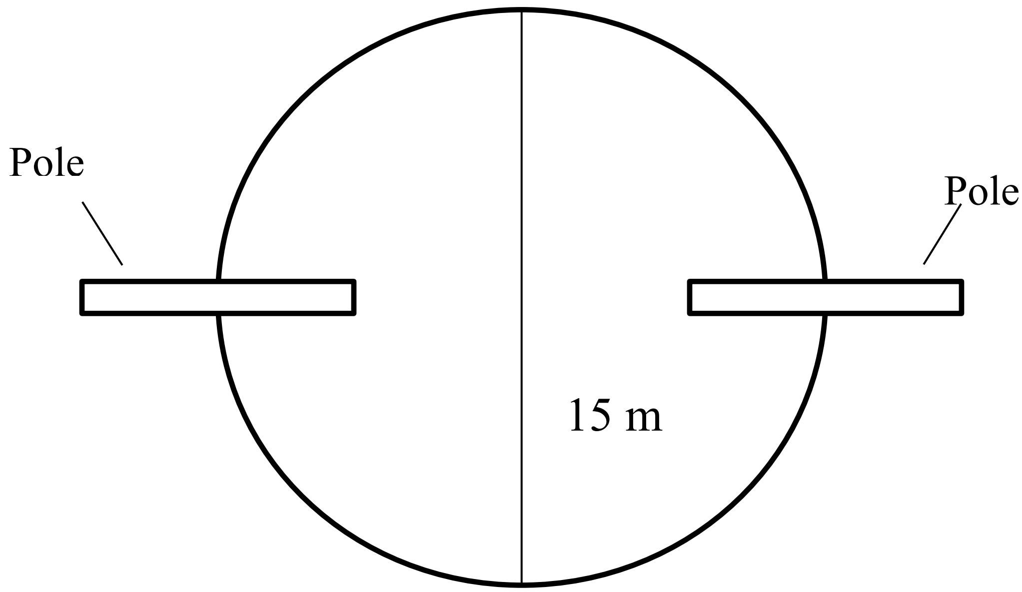
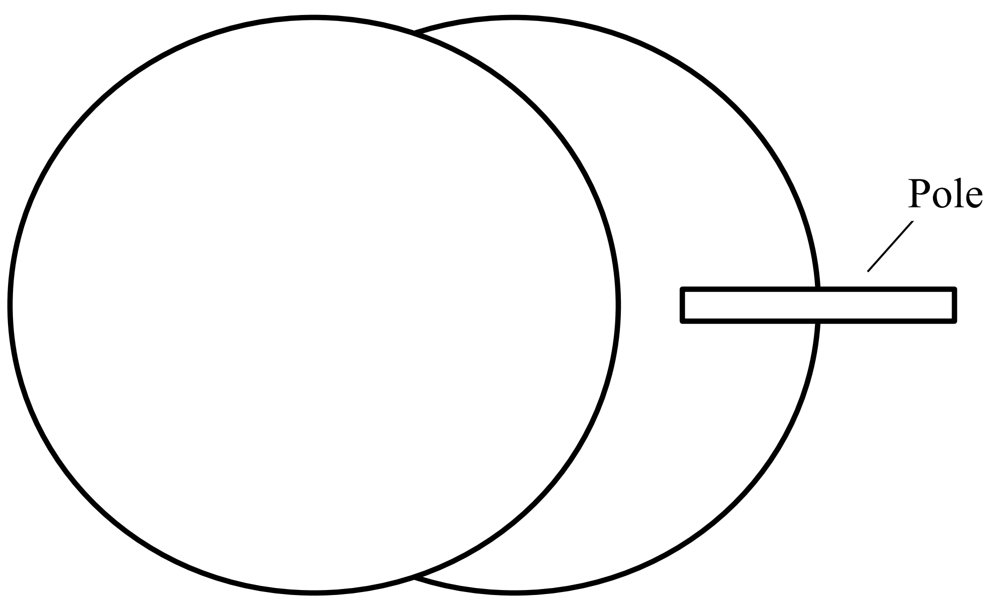


Figure 2. Diagram of exercise protocol two.



RESULTS AND DISCUSSION

Limitations

Due to limitations of horse availability, this study was performed using both mature, experienced horses along with immature, less experienced horses and would have been better if the age range was more standardized, or if the selection of horses for the treatment groups had been based on age group rather than the housing in which the horses were contained. Time constraints were also a limitation for this study. A longer exercise period could have been more beneficial and could have allowed for a switch back for treatment groups in order to compensate for the differences in age. The horses chosen for this study all had poor back conformation or lack of musculature in the loin region, thus creating a variation in body type causing a limitation for this study. A more uniform body type could have been more beneficial to the study. The method of measuring the back area in the horses, being that the method has not been validated, is another limitation to the study. There is room for human error with this method of measurement and needs to have further research done on the method in order to justify the measuring technique.

Data Analysis

During the first month of the experimental period all horses lost weight with the horses from treatment one losing significantly more weight than horses in treatment two. This may be due to the fact that all horse had been sedentary for a month prior to the

experiment and had no conditioning during that time period. Therefore, when the horses were put on strict exercise protocol weight loss was inevitable. Workload may also be a factor affecting the weight loss of the horses due to the temperature and percent humidity at time of exercising with the average temperature during exercising over the entire period at 26° C and humidity at 79%. Two of the five horses on treatment one lost an average of 30.6 kg (table 3). As a result the amount of the 14% concentrate these horses were fed was increased during the second period (table 3) during which time they regained some of that body weight. In comparison, horses on treatment two maintained their body weight during the six week experimental period, losing an average of 0.46 kg. This resulted in significant treatment differences ($P<0.05$) for change in BCS and rump fat and a trend ($P=0.063$) for a treatment difference in body weight change (table 11). Procedures used to measure forage intake and workload in this experiment do not allow for any further explanation for the difference in weight loss as both supplements were similar in digestible energy content and furthermore they provided a small percentage of the total caloric intake for these horses.

Both treatment groups lost belly and gaskin circumference, however, the changes were not significantly different between the treatment groups. Finally, for statistical analysis purposes the TES scores of A-D were converted to values of 1-4. Horses in treatment one, which lost the most weight, had an increase in mean TES value of 0.04 ± 1.1 as compared to treatment two which had a decrease in TES of -0.6 ± 0.56. This means that horses in treatment one went down in TES score and the horses in treatment two had a net increase in TES score. However, these changes were not significantly

different between treatment groups possibly due to the fact that the majority of change in score occurred for horse number five which lost 38.6 kg over the six week period.

Measurements for total back area are given for the three different positions measured (withers, back, and loin) by treatment group in table 12. During the six week period horses in treatment group one lost more total area in the withers, back, and loin (18.2, 18.6, 20.3 square cm) as compared to treatment group two which gained total area at the withers and loin (12.60, 2.88 square cm), and lost total area at the back (11.33 square cm). Furthermore, when these measurements were compared by area on the left side (table 13) vs. area of the right side (table 14) there was no significant difference due to protein supplement (treatment group) or from the beginning vs. the end of the experimental period for both treatments combined. Overall, as horses lost weight they also lost total area at the three positions measured. However, there is little information supporting that muscle was gained rather than fat being lost. There is potential for human error in the method used to determine back area. The method was suggested by Walker et al., 2016 and has not been validated. On the other hand as the horses lost weight back area decreased, thus demonstrating that this method has some potential. Further studies in which this type of measurement is couple with ultrasound examination to determine muscle area may prove useful.

In order to compare the change in total back area and left and right back area analysis of variance was conducted with body weight change, rump fat change, treatment, and position of the measurements as covariates (table 15). However, results of this

analysis indicated, in this study, none of these factors had a significant effect on the differences in measurements between the beginning and end of the study.

This study was designed using exercise protocols meant to affect specific groups of muscles, in this case the back, loin, and hindquarters. In order to determine if horses gained, lost, or maintained aerobic conditioning, horses were subjected to a standardized exercise test similar to one used in previous studies conducted by this research group (table 10). Heart rates measured during the test and recovery period are listed in table 16. There was no difference between the first and second SET for heart rate values at any time. When the effect of temperature, humidity, and % weight carried were used as covariates to compare differences in heart rate during the test and recovery period, there were no effects of treatment or SET. Heart rate values during recovery were not different.

When entered as covariates in the ANOVA analysis, both temperature and humidity had significant effect ($P<0.05$) on respiration rates at the end of the SET. Mean humidity was higher during the second SET compared to the first SET (table 17). Respiration rates were higher at the end of SET two as compared to SET one and five minutes in to recovery ($P<0.01$) and at 10 minute and 15 minute recovery ($P<0.05$). This was probably due to the higher humidity during SET two (table 18, 19). As humidity increases there is a decrease in the efficiency of evaporative cooling (sweating), thus due to their large lung capacity horses will rely to a greater extent on respiratory cooling. Webb et al., 2012 reported an increase in respiration rates during recovery from exercise when horses were on a diet containing of endophyte infected fescue seed. Finally, there was no difference in rectal temperatures at rest and after recovery (table 20). These

results show that horses did not gain aerobic conditioning over the experimental period. This was due to the type of exercise the horses were subjected to during the experimental period.

The use of a moderate quality mixed grass hay along with limited amounts of a concentrate at levels designed to maintain body weight when subjected to moderate exercise is a common practice for many horse's that are used for trail riding or "non-elite" level competition such as local horse shows rodeos, etc. Based on NRC requirements the hay and concentrate alone exceeded the needed crude protein intake (Appendix A). Therefore with the addition of the supplements both crude protein and lysine exceeded the requirements. As mentioned previously this protocol was copied from a previous report (Vineyard et al., 2013) and was meant to insure that if the exercise protocol was able to strengthen the musculature of the horse's back then neither protein quantity or quality would be a limiting factor. In this experiment the exercise did not improve musculature of the back as measure by the methods used. This is dissimilar to the report by Vineyard et. al. This may be because the exercise protocol was not strenuous enough or carried out for a long enough period of time to result in muscle development.

Table 11. Mean body weight difference (kg), BCS difference, rump fat difference (mm), gaskin difference (cm), belly circumference difference (cm), and topline evaluation score (TES) difference ± standard deviation, including P-values for both treatment groups.

	Treatment 1*	Treatment 2*	P- value
Body Weight	-19.98±17.01	-0.46±7.61	0.063
BCS	-0.800±0.570	0.100±0.418	0.001
Rump Fat	0.400±1.817	-2.00±3.39	0.012
Gaskin	-0.360±1.212	-0.660±0.999	0.837
Belly	-5.70±5.93	-2.200±1.255	0.544
TES	0.400±1.140	-0.600±0.548	0.839

*treatment 1 fed MFA Easy Keeper™
*treatment 2 fed Purina SuperSport™

Table 12. Mean whole back area (square cm) at withers, back, and loin, ± standard deviation for both treatments groups for horses fed different protein supplement.

Whole Back Area	Treatment*	Week 1	Week 6	Difference Week 1-6
Withers				
	T1	431.2±108.2	413.0±109.0	-18.2±23.9
	T2	372.7±45.0	385.3±58.0	12.60±16.61
Back				
	T1	332.7±52.3	314.2±34.3	-18.56±10.83
	T2	308.6±29.8	297.2±24.1	-11.33±21.78
Loin				
	T1	344.4±46.6	324.1±34.3	-20.3±27.0
	T2	308.4±19.88	311.29±6.45	2.88±15.24

*treatment 1 fed MFA Easy Keeper™
*treatment 2 fed Purina SuperSport™

Table 13. Mean back area left side only (square cm) at withers, back, and loin, ± standard deviation for both treatments groups for horses fed different protein supplement.

Left Back Area	Treatment*	Week 1	Week 6	Difference Week 1-6
Withers				
	T1	221.9±56.0	213.6±52.2	-8.26±7.67
	T2	188.52±22.05	198.1±38.5	9.54±21.39
Back				
	T1	170.4±30.7	161.5±31.2	-8.89±9.69
	T2	154.06±16.15	153.29±8.37	-0.77±19.09
Loin				
	T1	177.43±21.58	166.3±23.5	-11.12±11.55
	T2	155.43±13.54	163.40±7.44	7.97±16.25

*treatment 1 fed MFA Easy Keeper™
*treatment 2 fed Purina SuperSport™

Table 14. Mean back area right side only (square cm) at withers, back, and loin, ± standard deviation for both treatments groups for horses fed different protein supplement.

Right Back Area	Treatment*	Week 1	Week 6	Difference Week 1-6
Withers				
	T1	209.5±52.1	199.5±57.4	-9.99±18.73
	T2	184.3±25.6	188.2±29.4	3.83±20.73
Back				
	T1	161.76±22.20	153.3±22.8	-8.50±7.97
	T2	155.75±18.19	145.41±16.31	-10.33±10.95
Loin				
	T1	169.1±25.6	157.19±11.48	-11.88±19.47
	T2	153.45±7.10	149.06±6.20	-4.38±8.33

*treatment 1 fed MFA Easy Keeper™
*treatment 2 fed Purina SuperSport™

Table 15. P-values for anova analysis of treatment and covariate effect of horses fed different protein supplement on back area measurements.

	Whole Back Area	Left Area	Right Area
Body Weight Change	0.849	0.732	0.975
Rump Fat Change	0.614	0.594	0.793
BCS Change	0.863	0.686	0.782
Treatment	0.585	0.334	0.732
Position*	0.458	0.729	0.660

*withers, back, or loin

Table 16. Mean heart rates (beats per minutes) ± standard deviations at rest, during the SET, and recovery between SETs both treatment groups combined with SET.

Heart Rate	SET 1	SET 2
Resting	38.30±7.51	38.70±8.74
Series 1 Walk	62.45±9.12	61.15±11.06
Series 2 Walk	96.25±5.17	91.65±10.14
Series 3 Walk	97.60±5.99	95.15±9.26
Series 1 Trot	90.15±10.36	96.10±11.78
Series 2 Trot	95.00±7.37	97.05±7.32
Series 3 Trot	98.20±8.04	96.60±15.74
Series 1 Lope	122.80±12.91	123.55±12.96
Series 2 Lope	125.50±9.78	122.65±14.24
Series 3 Lope	124.15±11.22	128.20±15.35
End	128.70±9.36	127.30±14.06
1 Minute	80.50±9.29	82.00±7.50
5 Minute	64.50±5.50	65.80±8.87
10 Minute	60.10±8.10	61.20±9.89
15 Minute	56.30±7.90	57.20±11.25

Table 17. Mean temperature and humidity, SET, and speed horses traveled during the SET with treatments combined with SET.

	SET 1	SET 2
Temperature (C°)	28.22±0.44	28.83±0.88
Humidity (%)	63.48±3.160	79.48±4.75
Trot Speed (m/sec)	2.6957±0.2950	2.781±0.521
Lope Speed (m/sec)	4.552±0.438	4.241±0.353

Table 18. Mean respiration rate (expirations/m) for SET 1 and 2.

Respiration Rate	SET 1	SET 2
Rest	23.60±6.92	27.60±13.03
End	88.40±26.02^{c}	98.40±23.26^{d}
1 Minute Recovery	91.20±25.28	106.20±26.20
5 Minute Recovery	84.00±25.44^{c}	103.20±22.92^{d}
10 Minute Recovery	79.80±27.72^{a}	96.40±23.36^{b}
15 Minute Recovery	70.80±25.58^{a}	85.20±27.20^{b}

a,b different $P<0.05$
c,d different $P<0.01$

Table 19. Mean respiration rate (expirations/m) during both SETs for treatment group 1 and 2.

SET	Respiration Rate	Treatment 1*	Treatment 2*
S1			
	Rest	20.80±7.16	26.40±6.07
	End	88.00±33.6[a]	88.80±19.88[b]
	1 Minute Recovery	92.0±29.8	90.4±23.4
	5 Minute Recovery	89.6±29.6[a]	78.4±22.4[b]
	10 Minute Recovery	91.6±29.7	68.00±22.27
	15 Minute Recovery	76.8±31.7	64.80±19.47
S2			
	Rest	28.20±12.09	27.00±15.33
	End	100.8±24.6[a]	96.0±24.5[b]
	1 Minute Recovery	115.6±28.7	96.80±22.34
	5 Minute Recovery	110.40±21.28[a]	96.0±24.5[b]
	10 Minute Recovery	104.0±23.0	88.8±23.6
	15 Minute Recovery	96.0±28.3	74.4±23.9

a,b different $P<0.05$
*treatment 1 fed MFA Easy Keeper™
*treatment 2 fed Purina SuperSport™

Table 20. Mean rectal temperature (C°) for treatment group 1 and 2 for both SET.

SET	Rectal Temperature	Treatment 1*	Treatment 2*
S1			
	Rest	37.54±0.08	37.6±0.12
	15 Minute Recovery	38.53±0.25	38.24±0.32
S2			
	Rest	37.56±0.23	37.58±0.203
	15 Minute Recovery	38.68±0.36	38.3±0.63

*treatment 1 fed MFA Easy Keeper™
*treatment 2 fed Purina SuperSport™

CONCLUSION

The results of this study are in disagreement with a previous study by Vineyard et al., 2013 in which horses were exercise on a treadmill for 56 days and fed supplements similar to the ones in this study. With exercise protocols used in this experiment the feeding of the two supplements, MFA Easy Keeper™ and Purina SuperSport™, did not increase back area as measured by the procedures used. Total back area at the withers, back, and loin was not affected by the treatment group. This study did use a method of measuring the back that is not justified through research. However, the methods used did show some change in back area as horses lost weight and could be accepted as a type of measurements if further research is conducted using ultrasound imagines in conjunction with the flexible ruler. Horses from either treatment group did not gain or lose aerobic conditioning over the experimental period. Therefore in this study neither protein supplementation, nor exercise, affected the musculature of the back in horses as measured by the methods used. As a result of these findings the null hypothesis is accepted.

This study, along with other studies, has shown that protein supplementation has minimal effect on the musculature of the exercising horse. However, this study does not show any increase in the musculature of the back by implementing an exercise protocol while other studies have shown an increase in musculature of the back. This is likely due to the intensity and duration of the exercise protocol of this study. Further research is needed to develop exercise protocols which might increase back strength flexibility and in addition there is room for improvement in methods to verify this increase in strength.

REFERENCES

ACSM Position Statement. Joint position statement. 2000. Nutrition and athletic performance. Medicine and Science in Sports and Exercise. 12:1543-1556

Atherton, Philip J., T. Etheridge, P.W. Watt, D. Wilkinson, A. Selby, D. Rankin, K. Smith, and M.J. Rennie. 2010. Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. Am. J. Clin. Nutr. 92:1080-1088.

Flora H.F., D'Angelis, M.D.S. Mota, E.V.V. Freitas, G.C. Ferraz, A.R. Abrahao, J.C. Lacerda-Neto, and A. Queiroz-Neto. 2007. Aerobic training, but not creatine, modifies longissimus dorsi muscle composition. J. Equine Vet. Sci. 27:118-122.

Graham-Thiers, P.M., D.S. Kronfeld, K.A. Kline, D.J. Sklan, and P.A. Harris. 2000. Protein status of exercising Arabian horses fed diets containing 14% or 7.5% crude protein fortified with lysine and threonine. J. Equine Vet. Sci. 20:516-521.

Graham-Thiers, P.M., D.S. Kronfeld, K.A. Kline, D.J. Sklan, and P.A. Harris. 2003. Dietary protein and fat effects on protein status in Arabian horses during interval training and repeated sprints. J. Equine Vet. Sci. 23:554-559.

Hansen, Mette, J. Bangsbo, J. Jensen, M. Krause-Jensen, B.M. Bibby, O. Sollie, U.A. Hall, and K.Madsen. 2016. Protein intake during training sessions has no effect on performance and recovery during a strenuous training camp for elite cyclists. J. International Society of Sports Nutr. 13:9.

Hintz, Harold F. 1994. Nutrition and equine performance. J. Nutr. 124:2723S-2729S.

Haussler, Kevin K. 2009. Review of manual therapy techniques in equine practice. J. Equine Vet. Sci. 29:849-869.

Lamont, Linda S., A.J. McCullough, S.C. Kalhan. 1991. Comparison of leucine kinetics in endurance-trained and sedentary humans. J. Applied Physiology. 86:320-325.

Lamont, Linda S. 2003. Dietary protein and the endurance athlete. International Sports J. 39-45.

Liu, Yingying, X. Kong, G. Jiang, B. Tan, J. Deng, X. Yang, F. Li, X. Xiong, and Y.Yin. 2015. Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes. J. Animal Sci. and Biotech. 6:36.

Meyer, H. Nutrition of the equine athlete. Institut fur Tierernahrung, Tierarztlich Hochschule Hannover. 644-673.

Moore, Daniel R., D.M. Camera, J.L. Areta, and J.A. Hawley. 2014. Beyond muscle hypertrophy: why dietary protein is important for endurance athletes. Appl. Physiol. Nutr. Metab. 39:987-997.

NRC. 2007. Nutrient Requirements of Horses. 6th rev. ed. National Academy Press, Washington DC.

Oliveira, Katia de., R.V.G. Soutello, R. da Fonseca, C. Costa, P.R. de L. Meirelles, D.F. Fachiolli, and H.M. Clayton. 2015. Gymnastic training and dynamic mobilization exercises improve stride quality and increase epaxial muscle size in therapy horses. J. Equine Vet. Sci. 35:888-893.

Orton, R.K., I.D. Hume, and R.A. Leng. 1985. Effects of level of dietary protein and exercise on growth rates of horses. Equine Vet. J. 17:381-385.

Patterson, P.H., C.N. Coon, and I.M. Hughes. 1985. Protein requirements of mature working horses. J. Animal Sci. 61:187-196.

Paulekas, Rebecca, and K.K. Haussler. 2009. Principles and practice of therapeutic exercise for horses. J. Equine Vet. Sci. 29:870-893.

Qin, Chunfu, P.Huang, K. Qiu, W. Sun, L. Xu, X. Zhang, and J. Yin. 2015. Influences of dietary protein sources and crude protein levels on intracellular free amino acid profile in the *longissimus dorsi* muscle of finishing gilts. J. Anim. Sci. 6:52.

Rivero, J.L.L. 2007. A scientific background of skeletal muscle conditioning in equine practice. J. Vet. Med. 54:321-332.

Spillane, Mike, and D.S. Willoughby. 2016. Daily overfeeding from protein and/or carbohydrate supplementation for eight weeks in conjunction with resistance training does not improve body composition and muscle strength or increase markers indicative of muscle protein synthesis and myogenesis in resistance-trained males. J. Sports Sci. and Medicine. 15:17-25.

Vineyard, K.R., M.E. Gordon, P. Graham-Thiers, and M. Jerina. 2013. Effects of daily administration of an amino acid-based supplement on muscle and exercise metabolism in working horses. J. Equine Vet. Sci. 33:321.

Walker, V.A., C.A.Tranquille, S.J. Dyson, J. Spear, and R.C. Murray. 2016. Association of subjective muscle score with increased angles of flexion during sitting trot in dressage horses. J. Equine Vet. Sci. 40:6-15
.

Webb. Gary W., S. Demster, K. Minton, S.P. Webb, E. L. Walker, and B. Onyango. 2012. Effect of ergopeptines associated with tall fescue ingestion on recovery of horses subjected to standardized exercise tests. J. Equine Vet. Sci. 32:788-794.

Zhou, Ping, L. Zhang, J. Li, Y. Luo, B. Zhang, S. Xing, Y. Zhu, H. Sun, F. Gao, and G. Zhou. 2015. Effects of dietary crude protein levels and cysteamine supplementation on protein synthetic and degradative signaling in skeletal muscle of finishing pigs. PLoS ONE. 10(9).

APPENDICES

Appendix A. Covariate Effects on Heart Rates during Recovery

P-values for the effects of temperature, humidity, % weight carried, treatment and SET on HR at the end of the SET and during recovery

Heart Rate	Temperature	Humidity %	% Weight Carried	Treatment	SET
End	0.748	0.824	.083	0.939	0.810
1 Minute	0.578	0.555	0.732	0.907	0.573
5 Minute	0.623	0.735	0.478	0.901	0.736
10 Minute	0.545	0.580	0.311	0.857	0.583
15 Minute	0.788	0.795	0.373	0.941	0.828

Appendix B. Mean Heart Rates during SET 1 and SET 2 for both Treatment Groups

Mean heart rates (beats per minutes) ± standard deviations at rest, during the SET, and recovery by treatment group.

	SET 1		SET 2	
Heart Rate	Treatment 1	Treatment 2	Treatment 1	Treatment 2
Rest	36.80±6.65	36.80±8.79	37.20±5.22	40.20±11.80
Series 1 Walk	64.10±9.08	60.80±9.89	64.70±9.94	57.60±12.04
Series 1 Trot	92.00±12.90	88.30±8.16	98.50±15.57	93.70±7.42
Series 1 Lope	124.50±12.61	121.10±14.45	124.10±15.71	123.00±11.41
Series 2 Walk	97.50±5.89	95.00±4.62	94.40±10.98	88.90±9.57
Series 2 Trot	96.80±9.28	93.20±5.30	98.80±6.09	95.30±8.70
Series 2 Lope	125.80±12.32	125.20±7.94	121.90±16.25	123.40±13.81
Series 3 Walk	99.60±5.16	95.60±6.64	96.00±9.34	94.30±10.18
Series 3 Trot	101.50±7.53	94.90±7.83	103.20±6.66	90.00±20.11
Series 3 Lope	123.20±13.93	125.10±9.31	127.10±17.07	129.30±15.35
End	128.20±9.47	129.20±10.33	126.20±15.12	128.40±14.60
1 Minute Recovery	81.00±3.32	80.00±13.51	82.00±5.15	82.00±10.00
5 Minute Recovery	65.40±2.30	63.60±7.80	66.80±5.07	64.80±12.19
10 Minute Recovery	61.60.±5.03	58.60±10.81	62.40.±5.13	60.00±13.78
15 Minute Recovery	57.60±6.02	55.00±10.00	57.20±5.93	57.20±15.80

Appendix C. Crude Protein Intake

Estimated crude protein (CP) and intake by horse for period 1.

Horse	Body Weight (kg)	Forage	CP from forage (g)*	Grain (kg)	CP from Conc. (g)	Supplement (g)	CP from Supplement (g)	CP Required	Total CP intake
1	639	Pasture	959	2	280	390	125	981	1364
2	634	Hay *ad lib*	888	4	560	400	128	980	1576
3	616	9 kg Hay	648	3	420	370	118	946	1186
4	555	9 kg Hay	648	2	280	335	97	852	1025
5	507	Pasture	761	2	280	310	99	779	1140
6	618	Pasture	927	2	280	310	124	946	1331
7	564	Hay *ad lib*	789	4	560	280	112	866	1461
8	536	Pasture	804	2	280	270	108	823	1192
9	532	9 kg Hay	648	3	420	255	102	823	1170
10	525	9 kg Hay	648	4	560	260	104	806	1312

*All forages were Fescue, Orchard Grass, and Clover mix hay average 90% DM and estimated at a minimum of 8% CP as fed, pasture intake estimated at 1.5% of body weight and 10% CP, ad libitum hay intake estimated at 1.4% of BW and 8% CP

Appendix D. Lysine Intake

Estimated Lysine intake by horse for period 1.

Horse	Body Weight (kg)	Forage	Lysine forage (g)*	Grain (kg)	Lysine Conc. (g)	Supplement (g)	Lysine Supplement (g)	Lysine Required (g)	Total Lysine intake
1	639	Pasture	48	2	20	390	8.6	42	76.6
2	634	Hay *ad lib*	48	4	40	400	8.8	42	96.8
3	616	9 kg Hay	40	3	30	370	8.1	41	78.1
4	555	9 kg Hay	40	2	20	335	7.4	37	67.4
5	507	Pasture	38	2	20	310	6.8	33	64.8
6	618	Pasture	46	2	20	310	10.9	41	76.9
7	564	Hay *ad lib*	42	4	40	280	9.8	37	91.8
8	536	Pasture	40	2	20	270	9.5	35	69.5
9	532	9 kg Hay	40	3	30	255	8.9	35	78.9
10	525	9 kg Hay	40	4	40	260	9.1	35	89.1

*All forages were Fescue, Orchard Grass, and Clover mix hay average 90% DM and estimated at a minimum of .5 % lysine as fed, pasture intake estimated at 1.5% of body weight and .5 %, ad libitum hay intake estimated at 1.4% of BW and .5% lysine.