EFFECTS OF VERTICAL WHOLE-BODY VIBRATION ON SELECT BIOCHEMICAL MARKERS OF MUSCLE TURNOVER IN YEARLING HORSES

A Thesis

by

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

MASTER OF SCIENCE

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August 2017

Major Subject: Animal Science

ABSTRACT

This study was designed to determine the safety of vertical whole-body vibration (WBV) by measuring effects on select muscle biochemical markers in yearling horses on stall rest. Twenty yearling horses (17 ± 2 mon) were randomly divided into a split plot experimental design consisting of treatment (n=10) and control (n= 10) groups.

Horses were assigned uniform stalls by the Texas A&M University Horse Center and provided *ad libitum* access to water and trace mineral salt blocks. Horses were fed a diet for 100% of the NRC requirements for DE and 110% of the NRC requirements for protein, calcium, and phosphorus using 1.25% BW in Coastal Bermudagrass hay and 0.75% BW in pelletized concentrate.

The treatment group completed WBV on a vibration plate at 50 Hz 30 min per day 5 d per wk for 120 d. Serum was collected via jugular venipuncture in 6-mL lithium heparin tubes on D 0, 30, 60, and 120 before 30-min of turnout in on-site paddocks, and after turnout (control group only) or vibration (treatment group only). Only an initial blood draw was collected on Day 0 as a baseline value to serve as a covariate to which all mean values were adjusted. Samples were analyzed by Texas A&M University Veterinary Medical Teaching Hospital Clinical Pathology Lab for blood urea nitrogen (BUN), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), creatine kinase (CK), and lactic acid (LA) within 24 hr of collection. Statistical analysis was completed using the PROC MIXED procedure of SAS 9.4, and significance was set to $P \leq 0.05$.

There was no significant difference found serum BUN concentrations between the treatment and control groups across days and between pre- and post-treatment concentrations. No significant differences in serum AST and GGT concentrations were found between the experimental groups. Serum CK values were significantly different ($P = 0.0277$) between the treatment and control groups after treatment. There was no significant difference in serum LA concentrations between experimental groups. However, both groups each had a significant difference ($P < 0.05$) in LA values between D 30 and D 60 and between D 30 and D 120.

Elevated serum CK and AST concentrations are indicative of muscle metabolism during work. The presence of LA in the blood is indicative of anaerobic metabolism after high intensity physical exercise. Serum GGT is a reliable biomarker used to differentiate between liver disease and healthy muscle metabolism when measured alongside CK concentration. Vertical WBV of young horses on stall rest has proven to be a safe therapeutic tool with little to no significant alterations in skeletal muscle biochemical markers over time. Further studies using uniform muscle biopsies and hourly blood collections post-vibration are recommended for further understanding of the potential therapeutic applications of WBV.

DEDICATION

I would like to dedicate this thesis to my committee members, my supportive family, and the memory of Dr. Josie Coverdale. Without the personal investment of Dr. Coverdale, Dr. Martha Vogelsang, Dr. James Herman, Dr. Dennis Sigler, and my parents I could not have completed this work. Thank you for this opportunity.

ACKNOWLEDGEMENTS

I would like to thank my committee members, Dr. Dennis Sigler, Dr. Martha Vogelsang, and Dr. JamesHerman, in addition to Chelsie Huseman for their guidance and support throughout the course of this research. Chelsie served as a mentor and research partner and was willing to guide me through this project during the completion of her own study. I would also like to express my sincere gratitude to Dr. Chad Paulk, Dr. Jason Sawyer, Dr. Brandon Dominguez, Kailyn Capps, Haley Turner, and the numerous hard-working undergraduate students for their assistance with this work, as well.

In addition to the wonderful people that had hands-on experience with this project, I would like to thank the laboratory technicians at the Texas A&M University Veterinary Medical Teaching Hospital's Clinical Pathology laboratory.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a fulfilling experience.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

Part 1, faculty committee contributions

The work completed for the duration of this project was supervised by a thesis committee consisting of Dr. Dennis Sigler, advisor, and Dr. Martha Vogelsang of the Department of Animal Science and Dr. James Herman of the Department of Physiology & Pharmacology.

Part 2, student/collaborator contributions

The technicians of the Texas A&M University VMTH Clinical Pathology laboratory completed the serum analyses. The data analysis was completed by collaboration with Dr. Chad Paulk and Dr. Jason Sawyer of the Department of Animal Science.

All other work conducted for the thesis was completed by the student under the advisement of Dr. Dennis Sigler of the Department of Animal Science.

Funding Sources

This work was made possible by the financial contributions of Dr. Dennis Sigler in collaboration with Texas A&M University and Equivibe, Lincoln, NE. Equivibe was responsible for loaning the vibration plate to the project for the purposes of conducting our research.

NOMENCLATURE

AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CK	Creatine Kinase
CON	Control Group
GGT	Gamma-Glutamyltransferase
IACUC	Institutional Animal Care And Use Committee
LA	Lactic Acid
LDH	Lactate Dehydrogenase
TAMU	Texas A&M University
TRT	Treatment Group
WBV	Whole-Body Vibration

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Performance horses are equine athletes; they endure many methods of training in preparation for competition. Three-day eventing horses may have the most rigorous training regimen of all equine competitors: They require the endurance training for the cross-country event, high-intensity interval training (HIIT) to generate the power required for the over-fences event, and stamina for dressage. Racing Thoroughbred horses may require repeated bouts of short-distance (3/8 of a mile) at submaximal speeds with distance increasing over time. It is important to note that regardless of the training regimen, a horse will adapt to the training physically and physiologically. If training continues at submaximal potential (75-80% of maximum distance or speed) for a prolonged period of time, we begin to see signs of overtraining which could lead to breakdown of the athlete.

Some of the most commonly reported musculoskeletal injuries to performance horses include trauma to supporting soft tissue structures such as the deep digital flexor tendon (DDFT) or the suspensory ligaments. These structures can require several months of strict stall rest and/or controlled exercise upon sustaining even a minor injury. Over-exercising a horse can lead to joint inflammation (arthritis), inflammation of the synovial membrane within a synovial joint such as the knee (synovitis), cartilage breakdown, and chronic lameness. Overloading and overuse of muscles, tendons, and ligaments will lead to fatigue of these structures, significantly increasing the likelihood of injury.

1.2 Muscle Physiology

Skeletal muscles are connected to bones by tendons, which extend across a joint causing flexion or extension of a joint upon muscle contraction or relaxation. Skeletal muscles consist of hundreds of muscle fibers, each containing hundreds of myofibrils. However, the smallest functional unit of skeletal muscle is the sarcomere. A sarcomere is bordered on each end by Z discs, an A band that consists of thick and thin filaments, an I band at either end of the sarcomere, an M line and H zone at the center, and titin that connects thick filaments to the Z discs.

Conscious muscle contraction is initiated by the central nervous system (CNS), which then transmits a signal called an action potential via the alpha and gamma motor neurons to the muscle spindle fibers. These neurons then release a neurotransmitter called acetylcholine from vesicles within the end of the neuron, which crosses the synaptic cleft to the motor end plate of a muscle that causes the calcium release from the sarcoplasmic reticulum for muscle fiber contraction. Upon muscle contraction, the Z discs get closer together, and I bands and H bands become smaller. For these changes to occur, calcium must be available for cross-bridge cycling to take place between the heads of the thick filaments made up of myosin and the thin filaments containing actin, troponin, and tropomyosin. Calcium released from stores within the muscle binds to troponin to pull the troponin-tropomyosin complex away from the myosin cross-bridge binding site on the actin. Once this site becomes available, the actin-binding site of the myosin head may attach to actin, which triggers what is called a “power stroke” that pulls the thin filament inwards towards the center of the sarcomere. At the end of the

power stroke, adenosine triphosphate (ATP) is required to allow myosin to detach from actin so that another power stroke may occur to pull the thin filament further inward. During this single described power stroke cycle, other myosin heads are binding to actin to undergo power strokes at different times and different rates so that the muscle contraction is continuous until the muscle is told to relax by the CNS and calcium is returned to storage or the muscle is depleted of energy resources (ATP, creatine phosphate, glycogen, etc.).

Fiber types are differentiated by speed of contraction, time to fatigue, and metabolism. Type I fibers are referred to as slow-oxidative fibers; these fibers are responsible for slow, sustained contractions often found in postural muscles or endurance exercise muscles. Type I fibers contain low myosin-ATPase activity, but have high oxidative capacity which makes them highly resistant to fatigue. Long-distance runners or endurance racehorses have predominantly type I muscle fibers. Type IIa fibers are also oxidative fibers, but have a higher myosin-ATPase activity than type I fibers, which allows for faster contraction and lower resistance to fatigue. The last type of fiber is the type IIx fiber; this fiber type has the highest myosin-ATPase activity, lowest oxidative capacity, lowest resistance to fatigue, and fastest contraction speed of any of the fiber types. Type IIx fibers are glycolytic fibers and, therefore, contain a greater amount of glycogen than type I or type IIa fibers. Type IIx fibers are physiologically designed for high-intensity physical exertion. Olympic weightlifters and racing Quarter horses have a high percentage of type IIx fibers.

Beginning at birth and lasting through their first year of life, horses experience an increase in muscle length and cross-sectional area due to the insertion of new sarcomeres to myofibrils at either end of the muscle which is stimulated by the presence of growth hormone (GH), insulin, and thyroid hormone. In order for muscles to grow or increase in volume, they must increase in diameter of their fibers or undergo hypertrophy at the level of the myofibril or by increasing glycogen stores. Hypertrophy is the result of increased protein synthesis, satellite cell activation, or post-exercise micro-tears. Isometric contractions (muscle stretching), in addition to the presence of testosterone, induce splitting at the Z-discs of the sarcomere, leading to increased fiber diameter. Satellite cells are undifferentiated muscle stem cells that are activated by cytokines and growth factors, such as insulin-like growth factor-1 (IGF) and fibroblast growth factor-2 (FGF). IGF-I is responsible for both proliferation and differentiation of satellite cells, whereas FGF-2 stimulates satellite cell proliferation only (La Vigne et al, 2015).

Muscle atrophy is muscle wastage, reduction in muscle size, and reduced ability to generate force (Berg et al., 1997) that can occur upon incursion of a traumatic injury that causes denervation of muscle or any number of conditions or injuries that leads to disuse of a muscle or muscle group. Also, muscle dystrophy is a genetic disease that also leads to whole body muscle atrophy in addition to concurrent medical conditions. Disuse atrophy is most commonly seen in hospital patients on prolonged bed rest after a surgical operation or hospitalization for other health complications. In human models, prolonged disuse of muscle can result in substantial loss of muscle mass, strength, and circumference (Narici and de Boer, 2011). Ferrando et al. (1995) found human subjects

on a strict bed rest regimen for a period of 7 days lost at least 3% of thigh volume. Berg et al. (1997) found that human ICU patients on bed rest for 6 weeks experienced a 14 ± 4% reduction and 13 ± 9% reduction in the cross-sectional area and maximum torque production of the knee extensor muscle, respectively. Additionally, the previous study concluded that the loss of cross-sectional area and torque producing ability is not the result of muscle fiber type alterations. Kawahara et al. (2017) found that patients admitted to the ICU that were placed on bed rest for 72 h did not experience significant changes in limb circumference, however there was a significant decrease in limb circumference at 144 h post-admission with the greatest circumference reduction occurring in the lower limbs.

1.3 Muscle Adaptations With Age and Training

From birth, young horses will experience little or no change in oxidative capacity until they reach one year of age. Standardbred foals show a decrease in glycolytic capacity as they age, whereas Thoroughbred foals experience an increase in glycolytic capacity. Thoroughbred foals have greater alterations in their type IIa and IIx fiber ratios while their type I fibers remain unchanged. Overall, foals increase the ratio of type I and IIa:type IIx fibers during their first year of life. Alterations in oxidative and glycolytic capacity in addition to fiber composition are related to an increase in coordination, increase in proprioception, breed, gender, and genetics.

As training begins near their second year of life, young horses begin to see an increase in the type IIa to IIx fiber ratio, with a 30-70% increase in fiber size. However, Thoroughbred yearlings will have an increase in fiber size leading up to the

commencement of training as a result of aging. Unsurprisingly, aerobic training for endurance horses or long-distance race horses does not induce a profound increase in fiber area because aerobic training stimulates an increase in the type IIa:IIx fiber ratio and type IIa fibers have a smaller cross-sectional area. This reapportionment of fiber distribution is valuable in low-intensity, long-duration physical activity because a smaller cross-sectional area allows for more rapid oxygen transport through working muscle along with faster waste product removal from the same tissue.

Improved oxidative capacity effectively improves performance by making ATP formation more efficient, improving buffering capacity of lactate accretion, and allowing for improved free fatty acid oxidation to spare glycogen stores. The commencement of training increases the oxidative enzyme activity of skeletal muscle two-fold in addition to increasing the number of high-oxidative fibers and overall oxidative capacity within the first few months of training. However, glycolytic enzymes remain unaltered during training. Lactic acid dehydrogenase (LDH) is unlike other glycolytic enzyme activities in that its activity decreases with training, possibly due to improved static and dynamic buffering mechanisms within the muscle. Also, more high-oxidative type IIa fibers will convert to type IIx fibers with training. Creatine kinase (CK), phosphofructokinase (PFK), and LDH activity increase with intensive anaerobic training regimens.

1.4 Biochemical Markers

Several serum biochemical markers have been evaluated for efficacy in determining health status of the body. Creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-

glutamyltransferase (GGT) are the enzymes responsible for differentiating between muscle damage due to myopathies and exercise or liver damage. Blood urea nitrogen (BUN) is also a valuable blood marker used to evaluate the degree to which proteins are broken down in the body to produce urea via catabolic pathways or measure the degree of dehydration (hypovolemia). Recent studies have shown that female horses experience an increase in BUN, CK, and LDH post-exercise where male horses do not; however, both genders show an increase in AST and GGT post-exercise (Di Filippo et al., 2016).

Measuring BUN before and after physical exertion is a method for evaluating the kidney's ability to efficiently eliminate urea that is produced by the degradation of muscle protein during exercise. This test is popular among small animal veterinarians and human physicians to exclude the kidneys as a possible source of complication.

Creatine kinase (CK) is an enzyme found in the mitochondria and cytosol of cells in multiple forms. CK is responsible for the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by the transfer of one phosphate group to creatine to synthesize phosphocreatine. This reversible pathway forms a more stable energy reserve during muscle contraction (Brancaccio et al., 2010). During muscle contraction, CK is responsible for the conversion of creatine phosphate and ADP to creatine and ATP to fuel muscle contraction. Researchers have found that an increase in CK in response to exercise is highest after intense, endurance exercise; however, weight-bearing exercise proves to have a greater increase from baseline in enzyme activities. Trained human athletes experience less of an increase in CK when compared to untrained individuals, which suggests an adaptive response of the body's enzymatic release to exercise

(Horobagyi and Denhan, 1989). However, Koutedakis et al. (1993) found that the duration of physical exertion rather than physical fitness level of the individual had a stronger relationship to serum CK, AST, and ALT activities.

Lactate dehydrogenase (LDH) is the enzyme responsible for the conversion of lactate to pyruvate and vice versa while also reducing NAD+ to NADH in nearly all cells. Under anaerobic conditions, LDH favors the conversion of pyruvate to lactate in muscle cells after glycolysis. During the Cori cycle in the liver, LDH converts lactate to pyruvate, an energetically unfavorable reaction. Kobayashi et al. (2005) concluded that endurance exercise activities lead to a 100% increase in serum LDH activity, and levels remained elevated for approximately 2 wk post-exercise. In summary, muscle LDH response is correlated with muscle fiber composition (Costill et al., 1976).

Aspartate aminotransferase (AST) is the enzyme responsible for the conversion between aspartate and α-ketoglutarate to oxaloacetate and glutamate, which occurs between the mitochondria and the cytosol to form energy by transamination. Though this process produces viable energy for the cell, both its reactants and products are substrates for the tricarboxylic acid cycle, an aerobic energy-forming pathway within mitochondria. AST is primarily found in the cardiac and skeletal muscle cells, liver, and red blood cells. This enzyme is particularly useful in differentiating between healthy muscle activity and liver disease. An immediate increase in AST activity lasting for approximately 24 h after physical activity proportionate to the duration of the activity has been reported in previous research conducted by Lippi et al. (2008).

Gamma-glutamyltransferase (GGT) is an enzyme produced most notably in the liver, but it has been found in numerous other tissues as well. GGT is responsible for the conversion of amino acids, peptides, and water to glutathione by transfer of a gamma-glutamyl group. Physiological GGT levels are observed in the presence of elevated CK values in Duchenne muscular dystrophy patients, indicating the presence of a muscular disorder in the absence of liver disease and confirming the viability of GGT in distinguishing liver and muscle disease while CK is elevated (Rosales et al., 2008).

The aforementioned metabolites and enzymes, which represent some of the most valuable biochemical markers involved in the identification and differentiation of muscle degradation, were used in this study.

1.5 Whole-Body Vibration Treatment

Human medicine adopted therapeutic vibration plates long before the equine world; however, researchers have had limited results with the vibration plates. A study conducted in 2017 found only that the therapy method is safe and observed an increase in metabolism for patients on bed rest in intensive care units (Wollersheim et al., 2017). Early studies evaluating the effects of WBV in horses sought only to determine the physiological response of the musculoskeletal system after low-intensity vibration by measuring vitals, CK, cortisol, osteocalcin (OC), creatinine, myeloperoxidase activity (MPOa), and other biological indicators of muscle and bone metabolism (Carstanjen et al., 2013). Buchner et al. (2016) employed electromyography to measure muscle vibration and activity during whole-body horizontal vibration for a single bout of 10 min; these researchers did not observe any additional EMG activity while the horses

were on the plate, and deemed WBV an inadequate warm-up and exercise. A study evaluating the possibility of a vibration plate for therapeutic purposes in lame horses found no statistically significant improvements or alterations in the lameness status of horses undergoing treatment for 30 min twice per day, 5 d per wk for 60 d (Halsberghe, 2017). Neither Carstanjen et al. (2013) nor Buchner et al. (2016) employed a single uniform frequency of vibration for their studies.

Further investigation is necessary to determine the long-term alterations in serum biomarkers of muscle metabolism with a greater experimental population than in previous research studies. Furthermore, evaluation of the effects of vertical WBV for a greater duration of time per session and at a higher uniform frequency could provide insight into the effects of WBV as an alternative form of controlled exercise for injured horses on strict stall rest.

1.6 Goals and Objectives

The objectives of this study are to measure the changes in the serum concentrations over the course of the study as well as before and after treatment of the two experimental groups of the following biochemical markers:

1. Blood urea nitrogen (BUN)
2. Aspartate aminotransferase (AST)
3. Gamma-glutamyltransferase (GGT)
4. Creatine kinase (CK)
5. Lactic acid (LA)

The concentrations of these biomarkers were used to evaluate changes in skeletal muscle metabolism in order to determine relative safety of vertical WBV on skeletal muscle in yearling horses.

This study was a companion study conducted alongside researchers evaluating the effects of vertical WBV on bone density and turnover in yearling horses on stall rest.

2. PROBLEM

2.1 Muscle Atrophy

Prolonged muscle disuse due to traumatic muscle or nerve injury or reduced, restricted, or absent physical exertion leads to reduced muscle volume, circumference, and mass, otherwise described as muscle atrophy. During a period of prolonged disuse, the body will choose to reapportion energy that would normally be designated for the tissue structure(s) no longer in use to more energetically demanding structures or store excess energy in various forms. In the case of injured horses on stall rest, restricted movement is necessary for healing and prevention of further trauma or pain, however limiting the movement of the horse prevents muscle growth and allows the body to determine that certain muscle fibers, primarily type IIx fibers as they are the most energetically demanding, no longer require the same amount energy resources for maintenance. As long as muscles are not being used they will continue to decrease in size, mass, circumference, and maximum strength (Berg et al., 1997). Once a horse has completed its required stall confinement period, gradual reintroduction to physical exercise is necessary to prevent re-injury due to weak connective soft tissue structures and weakened skeletal muscles.

2.2 Owner Investment

From the moment of injury through treatment and rehabilitation, horse owners limit their horses' physical movement and stress to prevent further damage to the already injured structures. By confining their horses and preventing their use, horse owners

begin to invest more money in their care with less return on their investment. Veterinary diagnosis and treatment, feed bills, boarding, medications, possible surgical intervention, and treatment supplies (bandaging materials, salves, etc.) all require substantial monetary investment and time by way of the owner. Also during this time, the horse is not in training or competing, and, therefore, losing value. If the horse goes back to training or on to compete after injury and recovery, the risk of injuring the same structure or supporting structures is much higher. In addition, if the horse is placed on the market in the future, responsible buyers will have an equine veterinarian complete radiographs of vital structures (the spine, hocks, forelegs, etc.) to identify any prior injuries that the current owner has left undisclosed; identification of previously damaged musculoskeletal structures decreases the value of the horse due to the assumption that the injury may reoccur and require further investment for treatment and rehabilitation.

In the Thoroughbred racing industry, time that a horse spends not in training due to injury or any other medical complication is referred to as “wastage” (Bailey et al., 1997). Wastage encompasses financial and physiological variables, including money spent on treatment and rehabilitation and money lost by having to scratch a horse from a race with a large purse.

In summary, as long as the horse remains on stall rest unable to work, the owner is investing more money in the care and treatment of the animal to ensure its full recovery than the owner is receiving from the animal.

2.3 Animal Health and Welfare

While an injured horse is kept on strict stall confinement with or without restricted exercise, it is important that the horse is still allowed to exhibit natural equine behavior both inside and outside the confines of their stall to relieve stress and anxiety that can reduce the rate of healing. By reducing the rate of healing, the duration of confinement is extended and the horse is subjected to continued stress, forming a continuous cycle.

Injury and stall rest inhibit a horse's ability to exhibit natural behavior, allow for increasing stress and discomfort associated with the inability to express natural behavior, which prolongs the exposure to pain due to injury. These characteristics of stall confinement, even if it is for the horse's well-being, violates three of the Five Freedoms proposed by the United Kingdom's Farm Animal Welfare council in 1965 (Farm Animal Welfare Council, 2009). The Five Freedoms include:

1. Freedom from hunger or thirst
2. Freedom from discomfort
3. Freedom from pain, injury, or disease
4. Freedom from fear and distress, and
5. Freedom to express natural behavior.

By allowing for the cycle of limited exercise of natural behavior, increased stress, and prolonged pain to continue, horse owners are not attending to the overall welfare of the animal to the best of their abilities.

It is also important that we evaluate the safety of therapeutic tools such as the whole-body vibration plate for horses, young and old alike. Determining the safety of these devices for skeletal muscle and full-body health is an important factor in overall equine welfare.

2.4 Problem Summary

Researchers, veterinarians, and professionals in the equine industry are still seeking the most effective and efficient method to reduce time spent on stall rest, reduce financial instability and improve animal health and wellbeing during the healing process.

It is because of these tribulations in combination with promising human studies that equine professionals have embraced vertical WBV as a viable form of rehabilitation and physical therapy without concrete proof of its efficacy in equine models. In this study, we seek to answer the question of whether or not vertical WBV is a safe therapeutic tool for growing horses on extended stall rest by evaluating skeletal muscle biochemical markers that may indicate muscle damage.

3. MATERIALS AND METHODS

3.1 Experimental Design

Twenty horses 17±2 mon of age consisting of 16 mares and 4 geldings were selected from the horse herd owned by Texas A&M University's Animal Science Department and leased from private owners. All horses were of Quarter Horse or stock horse breeds. All horses were thoroughly evaluated by a veterinarian from Texas A&M University for overall health status prior to the onset of the study. Horses were maintained under the approval of the Texas A&M University Institutional Animal Care and Use Committee (AUP 2016-0090; appendix A) by the author, assisting researchers, Texas A&M University Horse Center staff, and undergraduate student assistants.

All horses were housed in 43.9 m^2 (3.66 m x 3.66 m) stalls at the Dick Freeman Arena, and were allowed 3 to 5 d for acclimation prior to the commencement of the study. Stalls were cleaned every morning as the horses were turned out or being vibrated. On weekends, horses remained in their stalls. Horses were turned out two at a time to on-site individual paddocks.

All horses were provided *ad libitum* access to water and trace mineral salt blocks for the duration of the study. Horses were fed a diet at 100% of the NRC recommendations for DE and 110% of the NRC recommendations for calcium, phosphorus, and protein for 120 d using Coastal Bermudagrass hay and pelletized concentrate (Producer's Cooperative, Bryan, Texas).

Horses were randomly and evenly assigned to one of two groups. The treatment group (n=10) stood on a vertical whole-body vibration (WBV) plate provided by Equivibe (Lincoln, NE) for 30 min per day 5 d per wk (Monday-Friday) with the vibration frequency set to 50 Hz. Horses in the control group (n=10) were returned to their stalls after turnout.

Each horse was weighed on a calibrated scale on D 0, 30, 60, 90, and 120, and individual weight measurements in pounds were recorded to ensure positive growth. Blood samples were collected on D 0 (before turnout) and D 30, 60, and 120 pre- and post-turnout (control group only) to vibration (treatment group only). The D 0 collection taken prior to turnout served as the baseline values for which all sample means were adjusted to as a covariate during statistical analysis. Serum samples were collected via aseptic jugular venipuncture using a 20 g hypodermic needle into a 6-mL lithium-heparin test tube, which was inverted 8 times prior to placement in a cooler with ice. Once all samples were collected, they were transported to the Texas A&M University Veterinary Medical Teaching Hospital's Clinical Pathology Laboratory for analysis within 24 h of collection. All blood samples were evaluated for creatine kinase (CK), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), lactic acid (LA), and blood urea nitrogen concentrations (BUN).

3.2 Data Analysis

The model was evaluated as a repeated measures or split plot design. Mixed model analysis was completed using the PROC MIXED procedure of SAS 9.4 (SAS,

INC., 2014), and significance was set to $P \leq 0.05$. SAS was used to determine the interactions, if any, between treatment, time, and day of each biomarker measured.

4. RESULTS

Table 1. Summary of Treatment Effect of WBV on Select Biochemical Markers.

	Day 0	Day 30		Day 60		Day 120		SE
Biochemical Marker[1]	**Before Only**	**Before**	**After**	**Before**	**After**	**Before**	**After**	
BUN (mg/dl)	16.00	13.66	13.82	12.67	12.37	16.27	15.42	0.34
AST (U/L)	347.80	293.90	292.6	302.65	302.45	308.85	292.45	9.08
GGT (U/L)	24.45	21.91	21.96	21.66	20.51	21.40	20.10	0.46
CK (U/L)	209.95	186.13	228.39	168.15	224.42	190.73	198.92	15.51
LA (mg/dl)	7.62	10.63	9.61	8.01	7.70	7.99	7.57	0.36

[1] Biochemical markers: Blood urea nitrogen, BUN; aspartate aminotransferase, AST; gamma-glutamyltransferase, GGT; creatine kinase, CK; lactic acid, LA.

Serum BUN concentration had significant day ($P < 0.0001$) and time ($P = 0.0172$) interactions with no treatment effect (Figure 1). The figure also elucidates the significant quadratic effect of day for BUN concentrations over the course of the study.

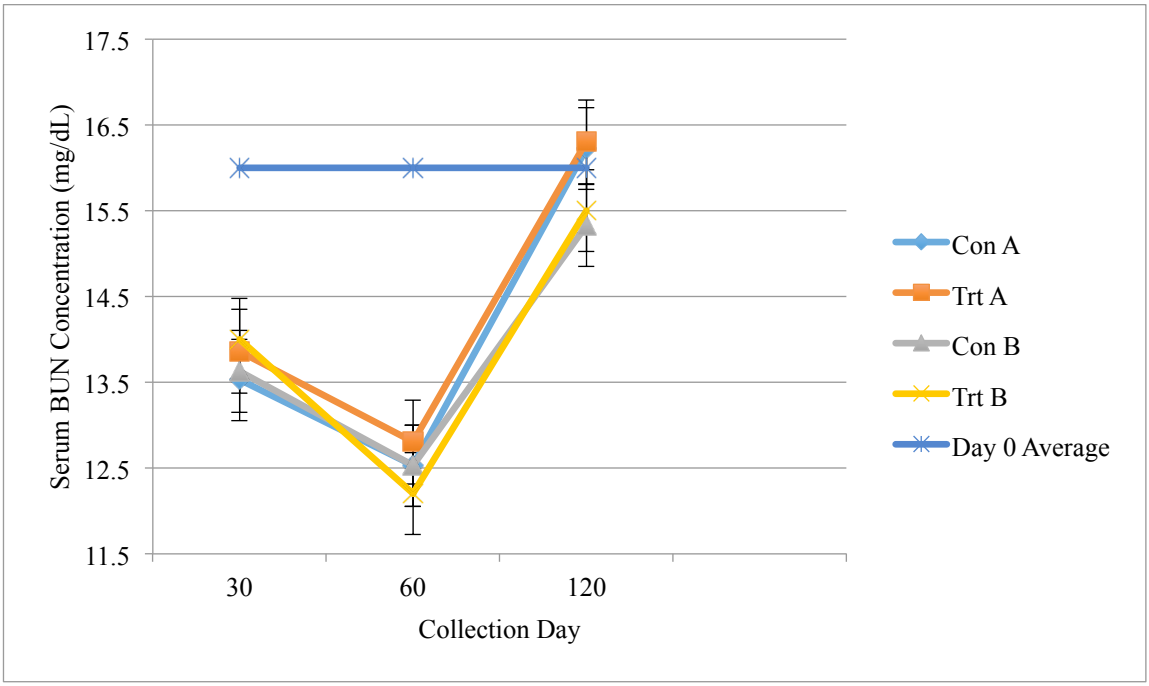

Figure 1. Effects of Treatment on BUN Over Time. ("Con"= control group, "Trt"= treatment group, "A"= before treatment, "B"= after treatment).

Average serum BUN concentration decreased for both the treatment and control groups from an average of 14.21 ± 0.26 mg/dl before turnout to 13.87 ± 0.26 mg/dl after completing treatment. However, no significant difference was found in serum BUN concentrations between the groups before and after treatment. Only a significant difference (P = 0.0404) was observed in horses of the treatment group before and after treatment. Additionally, serum BUN concentration increased in a quadratic trend for both experimental groups beginning at 13.76 ± 0.31 mg/dl on D 30, 12.52 ± 0.31 mg/dl on D 60, to 15.84 ± 0.31 mg/dl by D 120. No significant difference was observed

between the treatment and control groups for the duration of the project. The results show a significant difference ($P < 0.05$) between each day for each group.

Serum AST concentration had a significant interaction with time ($P = 0.0438$) in addition to a significant treatment-time interaction ($P = 0.0110$). The control group experienced a mild but not significant decrease in average AST values from 301.22 ± 7.41 U/L before to 287.69 ± 7.41 U/L after turnout over the entirety of the study, and horses within the treatment group did not experience a significant change in serum AST values (Figure 2).

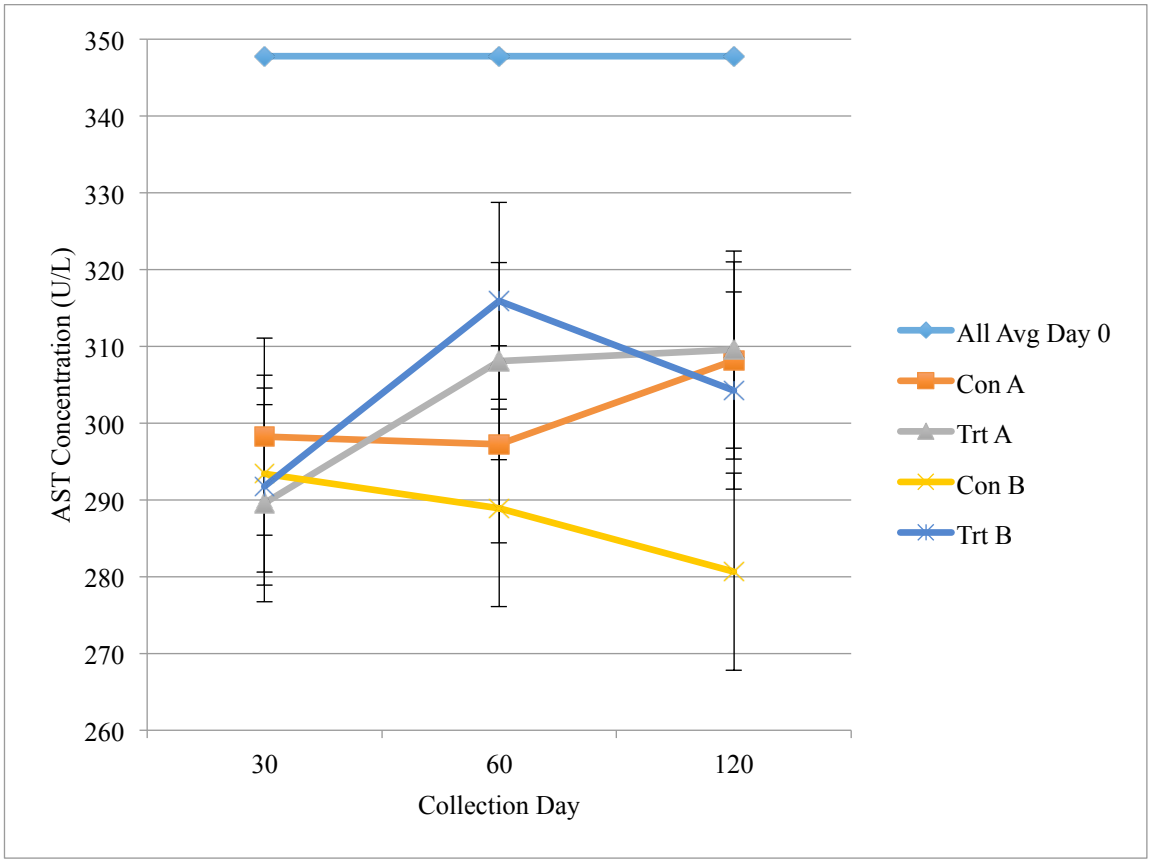

Figure 2. Effects of Treatment on AST Over Time. ("Con"= control group, "Trt"= treatment group, "A"= before treatment, "B"= after treatment).

The AST values of the horses within the treatment group averaged 302.38 ± 7.41 U/L prior to turnout and 303.98 ± 7.41 U/L after vibration. No significant difference was found in serum AST concentration between or within either experimental group before and after treatment.

Serum GGT concentrations revealed a significant interaction with time (P = 0.0331) in addition to a significant linear effect over the course of the study. However,

only the control group had a significant alteration (P = 0.0161) in serum GGT concentration before and after treatment. Additionally, there was a significant treatment-time interaction (P = 0.0349) without any significant alterations in serum GGT concentrations between the treatment and control groups (Figure 3).

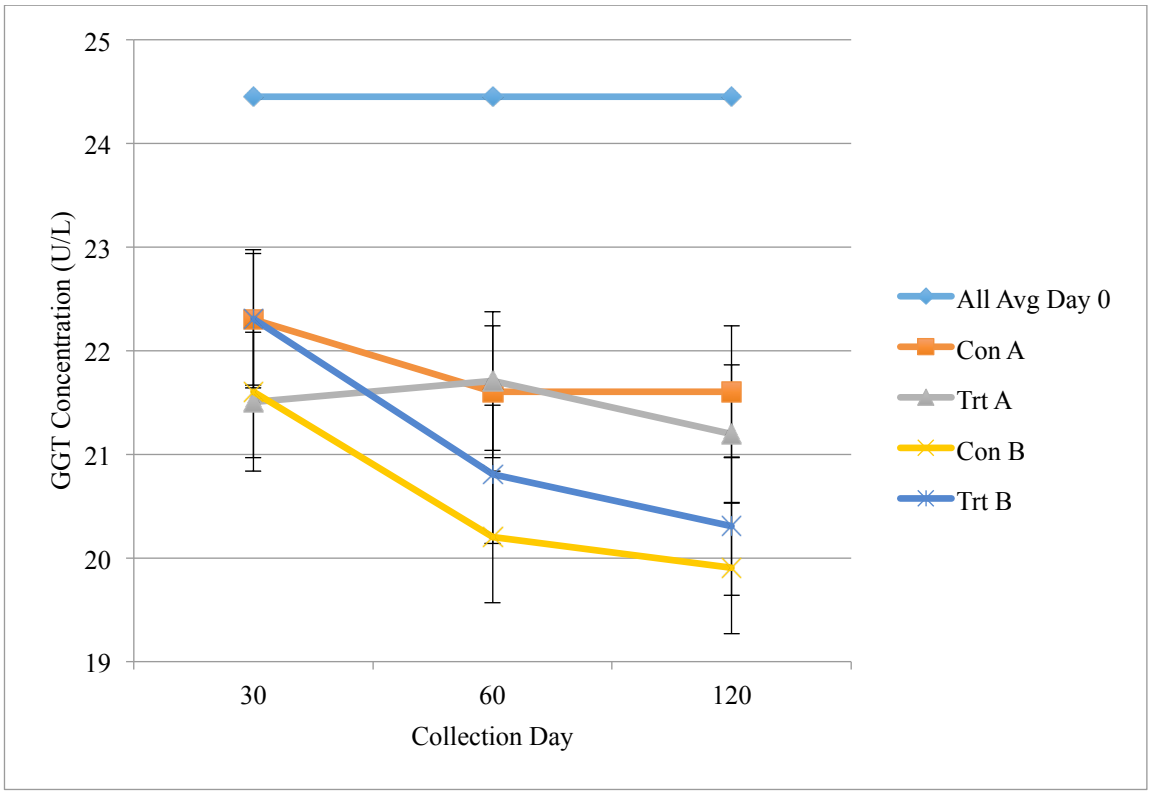

Figure 3. Effects of Treatment on GGT Over Time. ("Con"= control group, "Trt"= treatment group, "A"= before treatment, "B"= after treatment).

Horses in the control group had a greater reduction in serum GGT values compared to the treatment group from before to after treatment. Control horses averaged

21.84 ± 0 .37 U/L before turnout and 20.57 ± 0.37 U/L after turnout, whereas treatment horses averaged 21.47±0.37 U/L before turnout and 21.14 ± 0.37 U/L after vibration.

Serum CK concentration had a statistically significant treatment-day interaction (P = 0.0176) in addition to a significant interaction with time (P = 0.0012). A statistically significant difference (P = 0.0277) between the control and treatment groups was found in post-treatment serum CK concentrations in addition to the concentrations for the treatment group before and after treatment (P = 0.0034). Horses in the treatment group had increased serum CK concentrations (average of before and after treatment concentrations) from 194.91 ± 14.36 U/L on D 30 to 209.73 ± 14.35 U/L by D 120 while the horses in the control group experienced a decrease in serum CK concentrations from 219.60 ± 14.35 U/L on D 30 to 179.92 ± 14.36 U/L by D 120. However, the difference in serum CK concentrations between experimental groups pre- and post-treatment is not significant. Figure 4 demonstrates the effects of treatment, day, and time on serum CK concentrations.

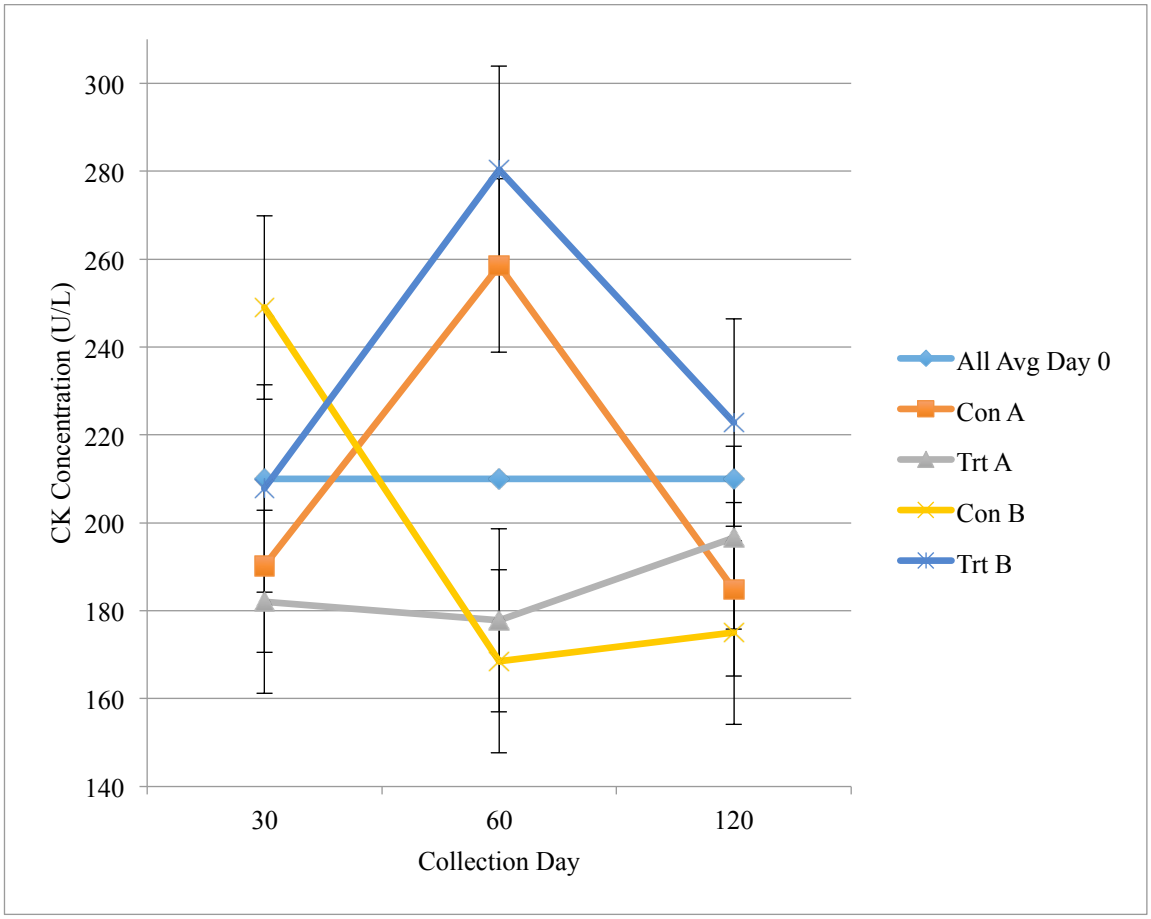

Figure 4. Effects of Treatment on CK Over Time. ("Con"= control group, "Trt"= treatment group, "A"= before treatment, "B"= after treatment).

Type 3 tests for fixed effects revealed both day ($P < 0.0001$) and time interactions ($P = 0.0147$) for serum LA concentration (Figure 5). No significant difference was found between the control and treatment groups between each collection day. However, significant differences ($P < 0.05$) were found for each experimental group from D 30 to D 60 and D 30 to D 120 only. Additionally, Figure 4 depicts the significant quadratic effect of day on serum LA concentrations.

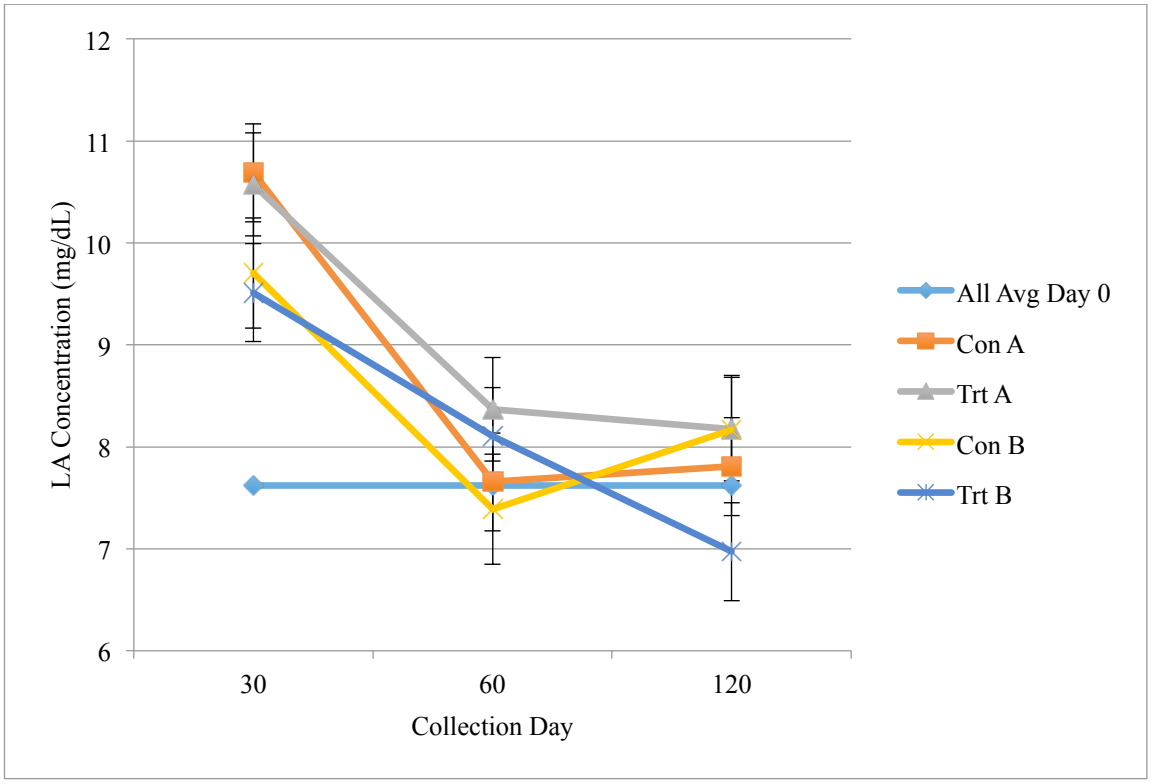

Figure 5. Effects of Treatment on LA Over Time. ("Con"= control group, "Trt"= treatment group, "A"= before treatment, "B"= after treatment).

No significant difference was found between experimental groups before and after treatment. However, there was a significant difference (P = 0.0297) for the only the treatment group before and after treatment. The control group experienced a decrease in serum LA concentration after treatment each day except for D 120. The serum LA concentration for the horses in the treatment group decreased each collection day.

Table 2. Summary of Day Effect of WBV on Select Biochemical Markers.

Biochemical Marker	Day 0	Day 30	Day 60	Day 120	Std Error
BUN (mg/dl)	16.00	13.76[a]	12.52[a]	15.84[a]	0.31
AST (U/L)	347.80	293.25	302.55	300.65	6.42
GGT (U/L)	24.45	21.93	21.08	20.75	0.33
CK (U/L)	209.95	207.26	196.29	194.82	11.01
LA (mg/dl)	7.62	10.12[bc]	7.86[b]	7.78[c]	0.25[abc]

[abc] Superscripts of the same letter indicate a statistically significant difference ($P < 0.05$) between two values.

Tables 3 and 4 describe the complete nutritional analysis of the Coastal Bermudagrass hay provided to the horses at the beginning of the study and the end of the study for comparison. The initial analysis was completed May 4, 2016. The final hay analysis was completed on September 16, 2016.

Table 3. Initial Coastal Bermudagrass Hay Nutritional Analysis.

Components[3]	As Fed	DM
% Moisture	7.30	-
% Dry Matter	92.70	-
% Crude Protein	9.90	10.70
% Adjusted Crude Protein	9.90	10.70
% ADF	39.40	42.50
% aNDF	69.50	75.00
% NFC	4.70	5.10
% TDN	51.00	55.00
NEL, Mcal/lb	0.34	0.36
NEM, Mcal/lb	0.42	0.46
NEG, Mcal/lb	0.19	0.21
Relative Feed Value	-	69.00
% Calcium	0.35	0.37
% Phosphorus	0.20	0.21
% Magnesium	0.16	0.18
% Potassium	0.65	0.70
% Sodium	0.37	0.39
PPM Iron	228.00	246.00
PPM Zinc	20.00	21.00
PPM Copper	6.00	7.00
PPM Manganese	238.00	257.00
PPM Molybdenum	0.60	0.60
Horse DE, Mcal/lb	0.74	0.80

[3] Components: Acid detergent fiber, ADF; neutral detergent fiber, aNDF; non-fibrous carbohydrate, NFC; total dietary nitrogen, TDN; net energy for lactation, NEL; net energy for maintenance, NEM; net energy for gain, NEG. Coastal Bermudagrass hay sample submitted for analysis May 4, 2016.

Table 4. Final Coastal Bermudagrass Hay Nutritional Analysis.

Components	As Fed	DM
% Moisture	8.00	-
% Dry Matter	92.00	-
% Crude Protein	14.00	15.20
% Adjusted Crude Protein	14.00	15.20
% ADF	32.20	34.90
% aNDF	62.50	67.90
% NFC	7.10	7.70
% TDN	52.00	57.00
NEL, Mcal/lb	0.42	0.46
NEM, Mcal/lb	0.45	0.49
NEG, Mcal/lb	0.22	0.24
Relative Feed Value	-	85.00
% Calcium	0.35	0.38
% Phosphorus	0.20	0.21
% Magnesium	0.18	0.2
% Potassium	0.69	0.75
% Sodium	0.46	0.50
PPM Iron	207.00	225.00
PPM Zinc	26.00	28.00
PPM Copper	11.00	12.00
PPM Manganese	170.00	184.00
PPM Molybdenum	0.40	0.50
Horse DE, Mcal/lb	0.81	0.88[4]

[4] Complete feed analysis for Coastal Bermudagrass hay submitted September 16, 2016.

5. DISCUSSION

Serum BUN is a measurement of the body's degradation of muscle protein during exercise and an indicator of kidney function. According to these results, WBV did not have a significant effect on BUN values between the treatment and control groups. However, the day of serum sample collection and the time at which the sample was taken each had significant effects on the measured serum BUN concentrations of both groups. The decline of this value after physical exercise indicates the absence of protein degradation or muscle breakdown in addition to healthy kidney function, which suggests that vertical WBV is not physically intense enough to produce detrimental skeletal muscle damage.

According to Lippi et al. (2008), serum AST increases in proportion to the duration of exercise. In this study, both the control and treatment horses had an overall decrease in AST values from before turnout to after turnout or vibration, respectively. However, the treatment-time interaction reported in the results indicates that the control group had a greater overall decline in average AST values after turnout compared to the treatment group. The absence of significant differences in serum AST concentrations suggests that neither turnout nor vertical WBV was intense enough or long enough to substantially increase AST values.

Elevated serum GGT is an indicator of liver disease only when combined with elevated serum CK. Both the treatment group and the control group experienced a decline in GGT in addition to a concurrent increase in CK values from before turnout to

after turnout or vibration. The control group had a greater decline in serum GGT values compared with the treatment group from before turnout to after treatment. The treatment group experienced a safe increase in serum CK concentration while the control group had an overall decrease in serum CK from D 30 to D 120. Elevated serum CK values are indicative of anaerobic activity. The reduced GGT values combined with elevated CK values indicate normal, healthy muscle and enzymatic activity in the absence of liver disease, supporting the hypothesis that vertical WBV is a safe therapeutic method for horses.

The presence of elevated blood lactic acid (LA) or lactate after physical activity is a strong indication that anaerobic metabolism has recently taken place. Both the control and treatment groups experienced a decrease in serum LA concentration, demonstrating the absence of physical exercise intense enough to overwhelm the body's natural ability to buffer any LA produced during turnout or vibration treatment. This could mean the vibration treatment was not of significant intensity in vibration plate frequency, long enough in duration on the plate, extensive enough in duration of the study, or simply ineffective as a method of anaerobic exercise.

6. CONCLUSION

Horse owners and riders are continuously looking for the most effective, efficient, and financially reasonable injury prevention and rehabilitation techniques. Unfortunately, not all of the methods and techniques the horse industry employs are proven effective for the designated purpose until long after they have gained infamy. In the case of the whole-body vibration plate as a tool for warm up exercise, physical therapy, rehabilitation, and treatment, previous research studies have found no evidence that suggests it is a viable tool for these purposes. In this study, we employed similar methods for measuring and testing the safety of vertical WBV as a therapeutic tool in addition to evaluating any changes in skeletal muscle biochemical markers for metabolism. Based on the results of the biochemical markers, in terms of muscle metabolism, vertical WBV is a safe therapeutic tool for growing, healthy horses. There is no evidence to suggest it may cause detrimental skeletal muscle damage based on the biochemical markers that were analyzed. Therefore, WBV can be considered a safe therapeutic tool for young horses.

Further research into this method of therapy may not be necessary. However, future studies should consider uniform muscle biopsy to monitor alterations in muscle fiber composition during periods of stall rest and vibration therapy. Evaluating heart rate, serum glucose and cortisol, and other valuable oxidative and glycolytic enzyme activities before, during, and after could add valuable insight to future academic studies on WBV in growing and mature equines both injured and healthy.

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APPENDIX A

IACUC AUP #: 2016-0090

DIVISION OF RESEARCH
Research Compliance and Biosafety

TEXAS A&M UNIVERSITY

May 24, 2016

MEMORANDUM

TO: Dr. Dennis H Sigler
ALRSRCH - Agrilife Research - Animal Science

FROM: Dr. John N. Stallone, Chair
Institutional Animal Care and Use Committee

SUBJECT: **Approval of Amendment**
AUP: IACUC 2016-0090
Title: The Effect of Vertical Whole Body Vibration on Bone Density in Horses.
Reference Number: 040293
AUP Approval Date: 05/19/2016
Expiration Date: 05/18/2019
Species: Equine

This is to inform you that the IACUC has approved the above-referenced amendment as follows:

- Caitlyn Hyatt has been added to this study.

The Committee thanks you for your efforts to keep the IACUC informed of any changes to your protocol. If we can be of any further assistance, please contact the IACUC office at 979.845.1828.

Best of success in your research endeavors.

Pc: Comparative Medicine Program

750 Agronomy Road, Suite 2701
1186 TAMU
College Station, TX 77843-1186

Tel. 979.458.1467 Fax. 979.862.3176
http://rsb.tamu.edu

APPENDIX B

PRODUCERS

96/1012

14% HORSE PELLET

For Foals, Mares and Mature Working Horses

GUARANTEED ANALYSIS

Crude Protein	(Min)	14.00 %	Phosphorus	(Min)	0.50 %
Crude Fat	(Min)	6.00 %	Copper	(Min)	45 PPM
Crude Fiber	(Max)	10.00 %	Selenium	(Min)	0.3 PPM
Calcium	(Min)	0.60 %	Zinc	(Min)	110 PPM
Calcium	(Max)	1.00 %	Vitamin A	(Min)	2,500 IU/LB

INGREDIENTS

Grain Products, Processed Grain Byproducts, Forage Products, Roughage Products (8%), Soybean Meal, Soybean Oil, Molasses Products, Ground Limestone, Monocalcium Phosphate, Salt, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin E Supplement, Vitamin B12 Supplement, Riboflavin Supplement, Niacin Supplement, Calcium Pantothenate, Menadione Sodium Bisulfite Complex, Folic Acid, Biotin, Thiamine Mononitrate, Pyridoxine Hydrochloride, L-Ascorbyl-2-Polyphosphate, Selenium Yeast, Cobalt Carbonate, Ferrous Sulfate, Ethylenediamine Dihydriodide, Manganese Sulfate, Zinc Sulfate, Copper Sulfate, Calcium Carbonate, Mineral Oil, Yeast Culture, Zinc Proteinate, Manganese Proteinate, Copper Proteinate, Dried Yeast Fermentation Solubles, Cobalt Proteinate, Brewers Dried Yeast, Dried Saccharomyces Cerevisiae Fermentation Solubles, Hydrated Sodium Calcium Aluminosilicate, Silicon Dioxide, Yucca Shidigera Extract, Calcium Propionate and L-Lysine.

FEEDING DIRECTIONS

Use the following table as a guide for the amount of feed to be fed per 100 pounds of bodyweight, according to designated horse type. Adjustments in these amounts should be based upon the quality of the forage (hay and/or pasture) the horse is consuming, size of the horse, and its degree of activity.

Horse Type	Daily Amt./ 100 lbs. Bodyweight
Mature Working Horse	0.5 to 1.25 lbs.
Lactating Mare	1 to 2 lbs.
Growing Horse	1 to 3 lbs.

In order to reduce the risk of colic, always feed at least 1-2 pounds of hay or forage per 100 pounds of bodyweight. Also, evenly space feeding times throughout the day, never feeding more than 8 pounds of concentrate per meal.

During periods of hot weather (daily temperatures of 90 degrees or higher) and/or heavy sweating, mix 2 oz. of an electrolyte supplement into the horse's daily ration.

Manufactured by
PRODUCERS COOPERATIVE ASSOCIATION
Bryan, Texas 77806

Net Weight 50 lbs. (22.68 kg) or Bulk

96 3 3/15/2013 MFG: