

STRESS RESPONSES IN HORSES USED  
FOR HIPPO THERAPY

by

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## ABSTRACT

Hippotherapy is increasingly used to address physical, occupational, speech, and psychological disabilities of clients, but research in this area is limited. The available research concludes that Hippotherapy provides many benefits to the client. Because Hippotherapy utilizes horses as part of the treatment team, it is vital to understand physiological wellness, potential sources of stress, and stress mechanisms in the therapy horse during the therapeutic process. Currently, there is some debate whether horses used for therapy are stressed in therapy situations. The North American Riding for the Handicapped Association (NARHA) recommends that therapy horses complete no more than three-consecutive sessions per day. The objective of this study was to measure potential stress mechanisms in the therapy horse during three-hour consecutive hippotherapy sessions. Five Quarter Horses from the Texas Tech University Therapeutic Riding Center completed two treatments, a control where measurements were taken at rest from 1200 h to 1700 h, and during Hippotherapy where measurements included pre-session (1200 h to 1300 h), three consecutive Hippotherapy sessions (1300 h to 1600 h) and post-session (1600 h to 1700 h) measurements. Heart rate was measured every three minutes, and blood samples were collected at the beginning of each hour to assess neutrophil counts, lymphocyte counts, neutrophil:lymphocyte ratios, monocyte counts, basophil counts, eosinophil counts, and cortisol levels. The behavior of each horse was assessed by a modified behavior scale by a NARHA instructor who was blinded to the study results. It was concluded that there was a horse difference for cortisol secretion. Horse by treatment interaction was significant for cortisol, heart rate, lymphocyte count, neutrophil-lymphocyte ratio, monocyte count, and basophil count. Treatment by time

interaction was significant for heart rate. Two of the horses may have been stressed due to higher cortisol and lymphocyte levels. One of these horses scored poorly on the behavior scale and was removed from the program due to increased agitation. However, the other horses were ideal based on desired behavioral traits. Both stress physiological measurements and the behavioral survey may allow therapeutic riding centers to better assess potential therapy horses and determine if current horses are being worked the appropriate amount or if adjustments need to be made.

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# CHAPTER I

## INTRODUCTION

The concept of horses helping humans is not a modern phenomenon. The horse as a healer appears in early mythology with the physician centaur Chiron, who had some patients that could not be healed. Chiron prescribed riding a horse for patients to lift their spirits.<sup>1</sup> Many medical writers in history used the horse in the treatment of disease. Some of these physicians include Oribasius of Sardis in 325 A. D., Galen in 130 A. D., Quellmaltz in 1735, Boerhaave in 1776, and Chassaigne in 1870.<sup>1</sup>

Equine-assisted therapy was pioneered in Germany.<sup>2, 3, 4, 5, 6, 7</sup> In Germany, Hippotherapy has been practiced by trained physiotherapists for over thirty years. They emphasized the physical and psychological effects on patients during Hippotherapy treatment. Benefits that were seen from Hippotherapy included increased muscle tone, improved trunk control, postural control, and increased confidence. Many physiotherapists stated that no other physiotherapy tool was as effective in regulating muscle tone as Hippotherapy and that the effects lasted longer than any other intervention.<sup>2</sup>

A woman named Liz Hartel from Copenhagen, Denmark was stricken with polio and wheelchair bound. She self prescribed riding to strengthen her muscles. Eventually Hartel won the silver medal for dressage in the 1952 Olympic Games in Helsinki.<sup>1</sup>

In 1954, Norah Jacques established the first riding facilities for the handicapped that served disabled children using ponies. This led to the establishment of the Pony

Riding for the Disabled Trust in 1964 located in Chigwell, England. The establishment of the Trust led to development of programs throughout the United Kingdom and brought professionalism and standardization of instruction and safety.<sup>1</sup>

In North America, therapeutic riding programs started to form in the 1960's. In Toronto, Canada, the Community Association of Riding for the Disabled was formed in 1960. In 1963 a similar program was established in Windsor, Ontario. In 1966, the Cheff Center was the first therapeutic riding center established in The United States in Augusta, Michigan.<sup>8</sup>

The North American Riding for the Handicapped Association (NARHA) was founded in 1969.<sup>8</sup> NARHA is the national organization for equine-assisted activities in the United States. NARHA is dedicated to promotion and improvement of riding for the handicapped, accreditation for therapeutic riding centers, development of standards for operating centers, certification for therapeutic riding instructors, distribution of information to the public, and development of continuing education for instructors and therapists.<sup>9</sup>

The Canadian Therapeutic Riding Association (CANTRA) is the national sports organization for therapeutic riding for disabled people in Canada. CANTRA was formed in 1980 to promote therapeutic riding, establish and maintain standards, to promote continuing education and research, and collaborate with the medical profession in Canada.<sup>10</sup>

### Study Objectives

Little is known about stress and horses used in Hippotherapy. NARHA has recommended limiting therapy horses to six hours a day and only three consecutive one

hour sessions per day. The objective of the current study was to evaluate stress of Hippotherapy horses during three consecutive sessions. Specific objectives of this study examined the comparison of horses during one hour pre-session, three consecutive Hippotherapy sessions, and one hour post-session to the same horses in a control setting (at rest). Measures included heart rate, blood cell counts, horse temperature, ambient temperature, and cortisol plasma levels. Measurements were correlated with behavior, which was assessed using a behavior score completed by a NARHA therapeutic riding instructor. The goal of this study was to determine a practical means to assess potential stress in therapy horses, to identify early warning signs of acute and chronic stress in these horses, and to provide potential recommendations for therapeutic riding centers involving the use of therapy horses.

## CHAPTER II

### EQUINE-ASSISTED ACTIVITIES

Equine-assisted activities are any activity with the horse which fosters cognitive physical, social, and emotional skills of the riders. These activities include Hippotherapy, therapeutic riding, equine-assisted psychotherapy, vaulting, sports riding or competition for a rider with physical, mental, or behavioral disabilities. “Although each area has its own unique goals and methods, the areas overlap and complement each other.”<sup>11</sup>

#### Hippotherapy

Hippotherapy literally means “treatment with the help of the horse” from the Greek word “hippos,” meaning “horse.”<sup>12</sup> Hippotherapy is therapy on horseback where the rider is influenced by the horse and its movement rather than controlling it. The movement of the horse manipulates the rider’s pelvis in a way that resembles the movement of the pelvis induced by the human walk.<sup>13</sup> This three dimensional movement of the horse’s hips and pelvis while the horse is propelling its hind legs at the walk provides movement challenge to the rider.<sup>11</sup> Some of the positions a client might use during a Hippotherapy session include but are not limited to facing forward, backward, supine, prone, or standing.<sup>11</sup> Therapists that treat clients during Hippotherapy are physical therapists, occupational therapists, and speech pathologists. If the therapist is not a certified NARHA instructor, a certified NAHRA instructor must be present to provide horse expertise to promote safety.<sup>11</sup>

This type of therapy is considered a direct medical treatment.<sup>12</sup> The therapist applies skill and knowledge to provide the rider with appropriate graded activities to

elicit adaptive responses that are favorable.<sup>11</sup> The intended outcome of participation in Hippotherapy is to achieve a higher level of function.<sup>11</sup> Hippotherapy provides intensive training of the trunk, coordination, reaction, balance, and alteration between tension and relaxation.<sup>9</sup> Some disabilities that are treated with Hippotherapy include amputations, autism, brain injuries,<sup>15</sup> stroke, cerebral palsy,<sup>16, 17</sup> Down syndrome, emotional disabilities, hearing and visual impairments,<sup>18</sup> learning disabilities, mental retardation, multiple sclerosis, muscular dystrophy, speech impairments,<sup>19</sup> spina bifida, spinal cord injuries,<sup>21</sup> and visual impairments.<sup>22</sup>

Physical benefits have been shown to improve respiration, circulation, balance, body metabolism, muscle strength, coordination, range of motion, self-esteem, self-image, communication, and interpersonal skills. Reduction of spasticity is also achieved through this treatment.<sup>23</sup> Improvements to posture, balance, and sensorimotor programming have also been documented.<sup>11</sup>

One benefit of Hippotherapy is motivation. The horse provides the rider with contact with an animal in a setting where therapy can take place outside of a clinical office. Another benefit of Hippotherapy is bonding. The rider faces a blend of emotions such as trust and respect from first meeting a horse, adoration of the experiences with the horse, and mastering any fear.<sup>9</sup> Communication is another benefit of Hippotherapy. Not only does the rider need to try to communicate verbally, but also through coordination of their hands and legs. Communication is displayed as emotions that do not block the communication connection between horse and rider. Self-esteem also increases as a result of the ability to achieve tasks through communication.<sup>9</sup> Sensitivity of the rider also improves with this type of therapy. The horse learns to accommodate the student's

consistently unsteady balance. In summary, Hippotherapy can initiate physical experiences that stimulate the student's balance mechanism, exercise his or her various body parts, give input to the joints, and strengthens the muscles.<sup>9</sup>

### Therapeutic Riding

Therapeutic riding is often used as a blanket term to refer to any equine-assisted activity for riders with disabilities. Therapeutic riding is defined as any mounted activity including traditional riding disciplines or adapted riding activities conducted by a NARHA certified instructor.<sup>14</sup> Therapeutic riding serves riders who also have disabilities. In many cases, these riders are higher functioning or function more independently than Hippotherapy clients. Often the rider has an active role of controlling the direction and cadence of the horse.<sup>11</sup> These activities contribute positively to the cognitive, physical, emotional, and social well-being of the riders.<sup>12</sup> Therapeutic riding provides benefits in different areas such as therapy, education, sport, and recreation.<sup>12</sup> Therapeutic riding provides many benefits for the client as well. The benefits seen in Hippotherapy are similar for therapeutic riding since both use the horse. Some of these benefits include vestibular stimulation, improved equilibrium, improved sitting, standing, walking balance, increased joint mobility, greater flexibility, increased gross motor coordination, increased strength, relaxation of spastic muscles, improved reflex time, improved cardiorespiratory function, and increased body awareness.<sup>9</sup>

Unfortunately, the population of disabled adults and disabled children who can benefit from therapeutic riding is far larger than the number of disabled adults and children who are fortunate to be able to participate in these well designed equine programs.<sup>1</sup>

Competition or sports riding is used to develop social skills while providing recreational therapy (Potter et al, 1994). Types of competition in this field include trail riding, Special Olympics, and Paralympics.

#### Equine-Assisted/ Facilitated Psychotherapy

Equine facilitated psychotherapy (EFP) is experiential psychotherapy involving horses. This type of therapy allows clients to enhance self-awareness and re-pattern maladaptive behaviors, feelings, and behaviors.<sup>24, 25</sup> The Equine Facilitated Mental Health Association (EFMHA) is a section of NARHA. EFMHA was founded over 30 years ago as a membership organization that promotes serving the needs of its members, mental health, education, and equine professionals. EFMHA promotes equine facilitated experiences that enhance psychosocial development, education, and growth.<sup>26</sup> Another professional organization for equine assisted therapy is The Equine Assisted Growth and Learning Association (EAGALA). This organization is not affiliated with NARHA, but provides certification for therapists and professional horsemen to promote professionalism and standardization in the field of equine-assisted psychotherapy.

Equine facilitated psychotherapy includes equine activities such as grooming, handling, longeing, riding, driving, and vaulting. Direct treatment is provided by a licensed mental health professional.<sup>27,25</sup> In a barn setting equine facilitated activities provide tools for building self-awareness, self-esteem, and self-responsibility. People who benefit from equine facilitated psychotherapy can have depression, low self-esteem, learning disorders, anxiety disorders, brain injury, sensory deficits, eating disorders, psychotic disorders, mood disorders, behavioral difficulties, schizophrenia, attention

deficit hyperactivity disorder, autism, receptive or expressive language disorders, personality disorders, Post Traumatic Stress Disorder, and substance abuse disorders.<sup>28</sup>

There is a difference between EFP and equine facilitated experiential learning.

EFMHA defines the two as:

Equine Facilitated Experiential Learning promotes personal exploration of feelings and behaviors in an educational format, while Equine Facilitated Psychotherapy both promotes personal exploration of feelings and behaviors, and allows for clinical interpretation of feelings and behaviors. Equine Facilitated Psychotherapy denotes an ongoing therapeutic relationship with clearly established treatment goals and objectives developed by the therapist in conjunction with the client. The therapist must be an appropriately credentialed mental health professional to legally practice psychotherapy and EFP. Equine Facilitated Experiential Learning falls under the heading of Equine Assisted Activities and may be conducted by a NARHA Instructor, an educator or a therapist. Experiential Learning refers to a style of learning that occurs when a person is interacting with the environment, including the people, animals, and situations involved. This involves learning by doing and may take place during a short period of time, such as during a workshop, or during regularly scheduled sessions.<sup>29</sup>

#### Types of Horses Used in Equine-Assisted Therapy Programs

A wide variety of horses are used in equine-assisted therapy programs.

Conformation of a therapy horse or potential therapy horse does not need to be perfect; however soundness of the horse is essential. No one type of horse can fill all the roles at a

therapeutic riding center. Any conformation faults must not affect soundness, health, and way of going. Age can vary widely for a therapy horse. A young inexperienced horse is generally not suitable. An aged horse may be experienced, but can have a shorter span of usefulness. Training time available, length of time horse will be useful, and costs are factors that need to be considered when determining a horse based on age. Health is another consideration, and any therapy horse or potential therapy horse needs to be in good health.<sup>12</sup> Smoothness of gait is not always a requirement. In some cases roughness of gait is desired for more sensory input of the rider. Generally the horse needs to move forward easily and freely, pick up his feet, and respond readily to voice commands. Horses also need to have a positive attitude, good manners, be reliable, be adaptable to different situations, and has had previous training with varied experiences.<sup>12</sup>

Currently there are no standards, national or international for trial periods of potential therapy horses, which are conducted after an initial training period. The recommendation of two weeks is considered to be sufficient, but four to six weeks allows horses to settle into their new environment and be exposed to different situations in which they will be working.<sup>12</sup>

### Scientific Research and Therapeutic Riding

In 1991, NARHA developed a five-year strategic plan to address the need for the development of an equine facilitated therapy research program due to the lack of scientific documentation of the benefits of therapeutic riding.<sup>13</sup> Currently there is a widening variety of scientific research on physical therapy and occupational therapy benefits for disabled people who participate in Hippotherapy or therapeutic riding.<sup>30,</sup>  
<sup>16,3,15 17, 18, 19</sup> Current research also supports Hippotherapy as an adjunct to traditional

physical and occupational therapy.<sup>11</sup> However, this area is in need of more current research and research to address a broader scope of different disabilities.

Currently the scientific research on horses used in therapy is limited to few published studies within the industry.<sup>31, 33</sup> Opinions vary in the industry on therapy horses and stress, but published scientific evidence is lacking. There is a great need of scientific research to further launch equine-assisted activities as a professional industry.

A study by Anderson and others<sup>31</sup> assessed different methods for selecting horses used in therapeutic riding programs. Both therapy (76 horses) and nontherapy (27 horses) horses were used from five therapeutic riding centers. A subjective temperament survey was completed for each horse by three therapeutic riding instructors or the most knowledgeable person at each site. Blood was collected to assess plasma cortisol, norepinephrine, and epinephrine levels. A reactivity test was then administered using three novel stimuli, a walking and vocalizing toy pig, popping a balloon, and opening of an umbrella. Behavior was then recorded and a reactivity score was assigned.<sup>31</sup> Reactions to the novel stimuli varied with popping of a balloon eliciting the least reaction and the opening of an umbrella eliciting the greatest reaction. There was poor agreement between instructors on the temperament surveys. No significant correlations were found among hormone concentrations, reactivity scores, and average reactivity scores. There were no significant correlations among total temperament score, average reactivity score, and hormone concentrations. Similarly, there were no significant correlations found among basal hormone concentrations, temperament, and reactivity. The authors concluded that it is hard to predict the suitability of a potential therapy horse based on temperament or

reactivity, and that programs need to be wary of the belief that a therapy horse does not adversely react to novel stimuli.<sup>31</sup>

Another study by Kaiser and others<sup>33</sup> assessed observable stress related behaviors of horses used in therapeutic riding programs. Fourteen horses were used in five groups with riders including able bodied riders, physically handicapped riders, psychologically handicapped riders, at-risk children, and special education children. The objective of this study was to determine what group of riders elicited higher stress or frustration in the horses. Equine behavior was observed and recorded for two minutes. Once all horses in the arena were observed one time, a variable scale for recording observations was used. This scale depended on how many horses were in the lesson at a given time. Seven behaviors were recorded that were indicative of stress, irritation, or frustration. These behaviors included pinning ears back, raising the head, turning the head left or right independent of the rider's control, tossing and shaking the head, lowering head carriage, and defecating. All horses were used no more than three consecutive hours, four days a week.

Kaiser and others<sup>33</sup> reported that the mean number of stress-related behaviors was variable for each riding group. No significant differences in behavior were found among riding groups, however the mean number of stress-related behaviors was higher for the horses when ridden by at-risk riders. The most common stress related behavior exhibited for all five groups was head movement. There were no significant correlations between horse age and mean number of horse behaviors observed.<sup>33</sup> Unfortunately, no other stress related parameters were used to measure stress other than observable behavior.

## Stress in Equine Studies

Stress requires an animal to make abnormal or extreme adjustments to its physiology or behavior in order to cope with adverse aspects of management or environment. These responses are the effects of stress. Stress cannot be avoided because demands will arise to provide the necessity to resist or adapt to the changing environment. Once a stressor is perceived by the animal, physiological changes occur within the body to help the animal resist stress. To be able to measure stress, parameters must be measured to indicate that the animal is responding to stress. Behaviors that are indicative of stress and physiological measures provided data that can be used as measures of stress.<sup>34</sup>

Some physiological responses to stress are increased heart rate, increased rate of breathing, increased cortisol concentrations, splenic contraction, increased muscle blood flow, increased metabolic rate, sweating, dilation of bronchioles, increased sensitivity of nerves supplying skeletal muscle, and increased mobilization of liver glycogen and fat stores.<sup>35</sup>

Stress elicited from increased confinement and isolation has resulted in increasing gradations of responses.<sup>36</sup> Stress is also elicited from transportation of the animal. Standards are being formed for transportation of horses going to slaughter in the United States. Equine transportation research has included transportation for breeding, showing, and racing. Hauling is a stressor for horses and can lead to salmonellosis, high heart rates, and changes in plasma ascorbic acid and cortisol concentrations.<sup>37</sup> Transport stress is composed of different influences that affect the physiological reactions horses have during transport. These influences include air condition, loading conditions, the horse's

orientation to the van or trailer, vibrations of the vehicle, confinement, density, head positions, and other potential stress sources.<sup>38</sup> In one study, plasma cortisol concentrations increased before transport, however this increase reflected hemoconcentration rather than transportation itself. The stress associated with hauling was not significantly greater than the experience of penned horses.<sup>37</sup> Another influence that effects stress during transportation is whether the horses received water before or during transport. Horses that did not receive water had cortisol levels increase throughout the transportation duration.<sup>39</sup>

Heart rate is affected by transport stress and it is also influenced by the driver's hauling ability. Horses hauled by an inexperienced driver had higher heart rates and more muscular movements for continual postural adjustments for balance preservation compared to horses hauled by an experienced driver. All groups had a discontinuous involvement of the sympathetic nervous system. The sympathetic nervous system was involved when there was fear during high stimuli, and then discontinued during halt phases as indicated by a placid status from horses.<sup>38</sup> Heart rate was also significantly elevated when horses are not watered before or during transport.<sup>39</sup>

#### Cortisol: A Measurement of Stress

Cortisol is a stress hormone. Cortisol concentrations increase during exercise<sup>40</sup> and cortisol has anti-inflammatory effects, and immunosuppressive effects after exercise.<sup>41</sup> After submaximal or maximal exercise, cortisol increases two- to threefold with a peak occurring 15-30 minutes after exercise. These levels return to pre-exercise levels within an hour. In untrained horses, cortisol levels were higher and took longer to clear from the blood than in trained horses.<sup>42, 35</sup>

Cortisol has a circadian rhythm in the horse which is highest in the morning and lowest in the evening.<sup>42, 43</sup> The circadian rhythm can be disturbed by removing the horse from its accustomed environment and placing it in a novel environment.<sup>44</sup>

Emotional stress also influences cortisol levels. An example of this is competition show jumping horses. These horses had higher basal resting levels than horses not competing.<sup>45</sup> This kind of emotional stress was also seen in transportation.<sup>38</sup> Cortisol does not protect against stress itself, rather it protects against the body's reactions to stress and prevents these reactions from being over-expressed and threatening.<sup>42</sup>

#### Cortisol and Age

Age also affects endocrine responses to exercise because there are changes in hormones associated with cardiovascular function, endocrine and paracrine factors, control of metabolic function, and stress hormones.<sup>41</sup> Cortisol production and secretion increases with age due to fewer receptors in the hippocampus.<sup>46</sup> Age-induced alterations in hormones coupled with cardiovascular function may be part of the mechanism of age induced declines in exercise capacity.<sup>47</sup>

#### Heart Rate: A Measurement of Stress

Heart rate adapts to exercise demands of the horse; however it also can also increase due to anxiety and apprehension. Heart rate response is proportionately larger in lower work loads because the heart rate levels off at a maximal level ( $HR_{MAX}$ ) when responding to high work intensity exercise. Horses that are trained have lower heart rate responses compared to less trained horses.<sup>48</sup>

Maximal heart rate is individualized and reached at variable exercise intensities, generally between 219-239 beats per minute. Once  $HR_{MAX}$  is obtained, heart rate cannot further increase. The work intensity that  $HR_{MAX}$  is obtained is also variable and correlated with maximum oxygen consumption ( $VO_{2MAX}$ ). Often work capacity is defined as the use of speed at a heart rate of 200 beats per minute ( $V_{200}$ ).<sup>48</sup> Submaximal heart rate is defined as less than 150 beats per minute. This is below the anaerobic threshold.<sup>35</sup>

One study by McCann and others<sup>49</sup> explored heart rate, respiration rate, and behavior between normal and highly reactive yearlings. Thirty-two Quarter Horse yearlings were grouped into two pens based on normal or nervous emotionality. Each yearling was then separated into a holding pen with a chute where respiration, heart rate, and behavior were recorded. Emotional behavior was rated as highly nervous, nervous, normal, or quiet. Higher heart rates were correlated with higher emotionality or nervousness. Normal yearlings had lower heart rate compared to nervous yearlings; however, the average respiration rate did not vary between normal and nervous yearlings.<sup>49</sup>

Another study, explored age-induced decline in aerobic capacity. Eighteen healthy unfit mares were grouped into three age categories, young, middle aged, and old. Measurements included  $HR_{MAX}$ ,  $VO_{2MAX}$ , oxygen pulse at  $VO_{2MAX}$ , and velocities at  $HR_{MAX}$ . All mares were measured during pre-training, during training, and at post-training with graded exercise tests on a treadmill. Mares were in training for three weeks, with a total of twelve weeks for the entire study. Results showed that aging results in the decrease of  $HR_{MAX}$  as compared to middle aged and young horses. There were no differences of  $HR_{MAX}$ ,  $VO_{2MAX}$ , oxygen pulse at  $VO_{2MAX}$ , and velocities at  $HR_{MAX}$  in

young and middle aged horses before or after training. Older horses had lower levels of  $HR_{MAX}$ , oxygen pulse, and  $VO_{2MAX}$  at lower velocities than young and middle aged horses. When all these unfit horses underwent training,  $HR_{MAX}$  was not altered in any age group. Training did increase all other parameters in all age groups. Older horses also had a longer  $VO_2$  recovery time before and after training.<sup>50</sup>

### Immunology: A Measurement of Stress

Immunology is the branch of science that focuses on the immune system and its responses. Blood cells have many functions including transporting hormones, waste products, nutrients, antibodies, ions, and other substances.<sup>51</sup> Neutrophils phagocytize bacteria by use of specific granules and modified lysosomes.<sup>52</sup> Neutrophils are an important part of nonspecific and specific cellular defense against invading microorganisms. Chemotaxis is one of the initial mechanisms where these cells are attracted to the site of infection.<sup>53</sup> Neutrophils are the most abundant and constitute about 60-70 percent of the blood leukocytes or white blood cells. The typical neutrophil count for horses is between twenty to sixty-five out of 100 total cells.<sup>51, 54</sup>

Eosinophils are granulocytes that defend against parasitic helminths and other inflammation mediators. The main products are modified lysosomes and specific granules which aid against parasitic infections.<sup>52</sup> Eosinophils constitute about 2-4 percent of blood leukocytes. Typically the count for eosinophils for horses is between one and four out of 100 total cells.<sup>51, 55</sup>

Basophils are a granulocyte that releases histamine and other mediators of inflammation. The main products of basophils are specific granules that contain heparin

and histamine.<sup>52</sup> Basophils constitute less than one percent of blood leukocytes.<sup>51</sup> The typical range of basophils for horses is between one and three out of 100 total cells.<sup>55</sup>

Lymphocytes constitute two cell types, B-lymphocyte and T-lymphocyte. B-lymphocytes generate antibody producing terminal cells, the main products are immunoglobulins. T-lymphocytes kill virus-infected cells with the main products being substances that kill and interleukins which control the activity of other leukocytes.<sup>52</sup> Lymphocytes can be large or small and constitute twenty to thirty percent of blood leukocytes, with a typical count between twenty and sixty-five out of 100 total in the horse. However, this range can be from two to ninety out of 100 total cells.<sup>51, 55, 56</sup> Exercise tends to increase lymphocyte counts as compared to pre-exercise levels.<sup>57</sup>

Monocytes are the largest agranular leukocytes which are young macrophages. Monocytes process antigens, phagocytize, and produce cytokines. The typical monocyte count for horses is one to six out of 100 total cells.<sup>51, 55</sup> The monocyte count increases after exercise compared to before exercise.<sup>57</sup>

Stress can cause immunosuppression, which leads to disease susceptibility. Exercise can transiently effect neutrophil functions and nonspecific defense mechanisms. However, exercise does not effect specific immune defenses. An increase in the neutrophil to lymphocyte ratio (N: L) coupled with increased cortisol concentration are indicators of stress.<sup>53</sup>

### Immunology and Age

Immune function decreases with advanced age which can lead to susceptibility to infection, tumor development, and autoimmune disease. Age-related changes in immune function can be exacerbated by stressors, which includes exercise. Corticosteroids also

are mediators of exercise-induced immune changes which can suppress the production of Interleukin-2 (IL-2) and other cytokines.<sup>46</sup> Total lymphocyte counts for horses typically decrease with age.<sup>58</sup> This decrease in lymphocyte counts with age are related to health, but can be a sign of age induced alterations of the immune system.<sup>58</sup>

Age is associated with alterations in lymphocyte subset T-cells (CD4+ and CD8+). In one study, older horses had lower monocyte counts post-training in a graded exercise test. Their lymphocyte count rose higher after exercise than middle aged and younger horses. The authors attributed the rise to age. Older horses had higher numbers of CD4+ (T-helper cell) lymphocytes at rest; however middle aged horses had comparable numbers to older horses. However, age did not have an effect of CD8+ (T-cytotoxic cell) lymphocytes, and training increased the number in all ages.<sup>59</sup> This suggests that exercise effects immunology of aged horses because healthy aged horses have been found to have a significant decrease in lymphocyte, T cells (CD4+ and CD8+) and B cell counts at rest.<sup>60</sup>

Like lymphocytes, typically monocyte numbers decrease with age. This also can be attributed to an age-induced decline of immunity. Increases in basophil and eosinophil counts for adult horses is typically due to changes in immunity due to exposure of parasites. Neutrophil counts typically remain unchanged in adult horses and is not affected by aging (Cebulj-Kadunc et al, 2003).

### Exercise Physiology and Age

Because many horses used in equine-assisted activities are older, it is important to understand physiology of the aged horse during work. Aged horses have similar submaximal oxygen consumption as compared to younger horses, however this is

significantly decreased if the aged horses (20 years or older) are unfit. During exercise, older horses fatigue at lower intensities exercise than their younger counterparts. They also have differences in aerobic capacity, lactate levels, and exercise capacity than younger horses.<sup>59</sup> At any submaximal speed older horses had to work at a higher relative velocity ( $\text{VO}_2$ ) than younger horses. Aging is also associated with physiological changes, decrease in relative maximal velocity ( $\text{VO}_{2\text{MAX}}$ ), decrease in maximal heart rate, and a decrease in muscle mass.<sup>61</sup> Clearly, decline in aerobic capacity and anaerobic power is due to the effects of aging on physiological functions.<sup>41</sup> It should be noted, however, that Hippotherapy only involves the horse at a walk or trot so anaerobic thresholds are never approached. Unfit older horses cannot thermoregulate as well as younger unfit horses. This is due to the decline in the cardiovascular system, specifically a compromise in cardiac filling pressure and cardiac output during exercise.<sup>50</sup> The insulin-like growth factor (IGF-1) concentrations decline with age thus altering immune function, endocrine function including cortisol, metabolic function at rest and exercise. A lowered cortisol response may be attributed to age-related alterations in T-cell function and immunosenescence.<sup>41</sup>

CHAPTER III  
METHODOLOGY AND RESULTS

Materials and Methods

General

Five Quarter Horses, four geldings and one mare, from the University Therapeutic Riding Center (UTRC) at Texas Tech University, were used in the study. The range of age for the five horses was eight to twenty-seven years (Table 3.1). Hippotherapy experience for all five horses spanned nine months to seven years (Table 3.1). The mean body condition score<sup>62</sup> of the horses was six (Table 3.1). The mean weight was 561.8 kg measured by a MTI-500 Weight System (Equine Racing Systems Inc., Washougal, WA, Table 3.1). All sessions were conducted inside the covered enclosed arena (36.57 meters wide x 76.20 meters long) of the Texas Tech Equestrian Center, Wolfforth, TX. All doors leading into the arena were closed. The horses were housed in the University Therapeutic Riding Center two-acre pen with three-sided shelters (6.09 meters long x 3.65 meters wide). Water and hay were provided *ad libitum*. All equine management and care procedures and the study protocol were approved by the Texas Tech University Animal Care and Use Committee.

The design of the study consisted of monitoring five horses as they completed two treatments, a five-hour Control treatment and a three-hour Hippotherapy treatment included a one-hour pre-session and a one-hour post-session measurement period. Horse responses in three consecutive Hippotherapy sessions were evaluated, because NARHA recommends a limit of three consecutive therapy sessions per day for each therapy horse.

The control treatment was conducted at rest. Horses were tacked up with saddle pads, single handle surcingle, English leather breast collars and halters and tied to a metal fence ambient the arena located next to the mounting ramp. This area was used as horses waited for their next session. Each horse completed one five-hour control treatment. The control and Hippotherapy treatments did not take place on the same day. The control treatments started at 1200 h and ended at 1700 h. Heart rate was measured every three minutes or 20 measurements per hour, with 100 measurements total per horse. Blood was collected hourly by jugular venipuncture for cortisol and blood data, ambient temperature, and rectal horse temperature were taken at the beginning of each hour; six times total (Table 3.2).

The second treatment monitored the horses during Hippotherapy sessions. All horses completed the Hippotherapy treatment one time. The riders were pediatric clients of the University Medical Center and current Hippotherapy clients of the UTRC. These children had various disabilities requiring two side-walkers and a horse leader for therapy. However, the level of involvement in each child's disability was variable. The Hippotherapy treatment started at 1200 h. Pre-session measurements occurred from 1200 h to 1300 h to establish baseline measurements of heart rate, horse temperature, and cortisol concentration. Blood was collected for cortisol measurement by jugular venipuncture. At 1300 h the first Hippotherapy session started. This session began when the child arrived, was fitted by a physical, occupational, or speech therapist with a riding helmet and cloth safety belt with one handle on each side. Each child was placed on the horse at the mounting ramp by a therapist. The mounting ramp was made of metal and consisted of a 10.97-meter long ramp for wheelchairs on the left side. Stairs lead up to the

highest portion. The bottom was an incline for wheel chairs to reach the top. To the right of the ramp was a space for the horse to move up next to the ramp. To the right of the ramp where the horse was placed is a three-foot space for the horse to enter a metal mounting block. The left sidewalker stood on the uppermost portion of the ramp, where the client and therapist were located. The right sidewalker was on the mounting block. The horse leader was in front of the horse. Once the child had mounted, the child, horse-leader, and side-walkers walked into the arena with the therapists to begin the Hippotherapy session. Both the right and left sidewalkers held the cloth handles located on the side of the belt around the waist placed by the therapists. Heart rate measurements were taken throughout the Hippotherapy treatment session, twenty measurements for the session. Each action of the session was also noted (ie. trotting, halting, and participating in individualized therapy activities with the therapist). Each session lasted fifty minutes. At 1350 h the child was dismounted and led out of the arena by the therapist. The horse was lead out of the arena once the child was in the custody of their parents. Ambient temperature, horse temperature, and blood were collected for 1400 h. The horse was tied back to the same metal fence located in between the mounting ramp and the arena. At 1400 h the second Hippotherapy session started. The same process occurred for session two. The rider was mounted on the horse using the mounting ramp and then led into the arena for the session to commence. Twenty heart rate measurements were recorded during the session, as well as each action of the therapy. At 1450 h the rider was dismounted with the aid of a therapist and escorted out of the arena. The horse was then taken out of the arena for temperature, horse temperature, and blood collection and then retied. At 1500 h the last Hippotherapy session started. The same procedure for mounting

was used with the mounting ramp and then moved into the arena for the session to commence. Twenty heart rate measurements were recorded, as well as each action. The session ended at 1550 h where the rider was dismounted and escorted out of the arena by a therapist. At 1600 h, one hour of post-session measurements were conducted. Heart rate and actions were recorded until 1700 h where blood, temperature, and horse temperature were collected for the last time (Table 3.2).

### Temperature

Ambient air temperature was measured inside the arena and recorded six times, once at the beginning of each hour with a Tempi Watchworks (Disputanta, VA) hanging thermometer. Horse temperature (degrees Celsius) was taken rectally with a Vicks brand V900 digital thermometer (New York, New York) at the beginning of each hour for a total of six measurements.

### Blood Collection and Samples

Blood was collected hourly by jugular venipuncture with Vacutainer Precision Glide brand blood collection needles at the beginning of each hour for a total of six times. Blood samples were stored in BD brand Lithium Heparin blood collection tubes (7 mL). Blood collection started at 1200 h and ended at 1700 h. All session measurements were conducted at the beginning of each hour from 1200 h to 1700 h to reduce the effect of diurnal variation in cortisol. All blood samples were stored on ice and transported to the Texas Tech University Physiology Lab.

Whole blood slides were made at the lab for each samples collected for each hour. Slides were stained with Protocol Hema 3 fixative (Fisher Scientific, Pittsburgh, PA) for one minute, and then dried. Once slides were dried, they were dyed with Protocol Hema 3

Solution 1 (Fisher Scientific, Pittsburgh, PA) for one minute immediately, then with Protocol Hema 3 Solution 2 dye (Fisher Scientific, Pittsburgh, PA) for thirty seconds. Slides were rinsed with distilled water. Once slides were dried, lymphocyte, neutrophil, monocyte, eosinophil, and basophil cells were counted using a Bausch and Lomb microscope (Rochester, NY, model number 402082) and Fisher Scientific brand manual cell laboratory counter.

### Heart Rate

Heart rate was measured by a Polar Equine Heart Rate Monitor S-610 (Polar Electro Oy, Kempele, Finland). Heart rate was recorded every three minutes. The heart rate monitor consisted of 2 electrodes. The positive electrode was placed under the left side of the withers under the saddle pad. The negative electrode was placed in a ventral position under the girth and secured with a provided strap and clip. The transmitter was placed on the neck where the shoulder slopes and attached by a provided strap and clip. Both electrodes and transmitters were connected by one wire and placed to make direct contact to the horse using Priority Care 1 non-spermicidal sterile lubricating jelly (First Priority, Inc., Elgin, IL). The heart rate monitor watch was placed on the breast collar where the leather strap attaches the breast collar to a circular metal ring on the surcingle. The heart rate monitor continuously monitored heart rate. Once all heart rate measurements were completed, the information was downloaded to a computer.

### Cortisol Assay

The blood samples were weighed using Ohaus Harvard Trip Balance (Ohaus Corporation, Pine Brook, New Jersey) then placed in the Beckman Model TJ-6 centrifuge (Beckman Coulter Inc., Chaska, MN) for fifteen minutes on speed seven for plasma

separation. Plasma was removed by an Eppendorf Research 1000  $\mu$ l pipette (Westbury, New York) with Fisherbrand Redi-Tip 1000  $\mu$ l pipette tips (Fisher Scientific Company, LCC, Pittsburgh, PA) and placed in Nunc Cryotube™ 1,8  $\mu$ l vials. Samples were then frozen in the lab freezer. Cortisol samples were assayed with an ImmuChem™ coated tube Cortisol  $^{125}$ I (Iodine-125) RIA (radioimmunoassay) in vitro kit (MP Biomedicals, LLC, Orangeburg, NY). The procedure of the assay included pipetting 25  $\mu$ L of serum standards, controls, and cortisol samples into the designated provided coated polypropylene tubes. Then 1  $\mu$ L of  $^{125}$ I tracer was added to all tubes, and each tube was placed in the vortex mixer. All tubes were incubated for 45 minutes at 37°C in a water bath. All samples were decanted, and counted using a gamma radiation counter (Packard Cobra II, GMI, Inc., Ramsey, MN).

#### Behavioral Survey for Head NARHA Instructor

A behavioral survey adapted from Anderson and others<sup>31</sup> was distributed to the Texas Tech UTRC Head Instructor. This instructor was blinded to the study and results. The survey was completed based on previous knowledge and interaction with the five Hippotherapy horses used in this study (Table 3.3).

#### Statistical Analysis

Analysis of variance (ANOVA) was conducted using the Generalized Linear Model Procedure.<sup>32</sup> Analysis included the independent variables horse by treatment interaction, the dependant variables were cortisol, neutrophil, lymphocyte, monocyte, eosinophil, basophil, ambient temperature, horse temperature, and heart rate. The Generalized Linear Model Procedure was used to analyze each dependent variable with time by treatment interaction as another independent variable. Then control and

Hippotherapy treatments were analyzed as the independent variables with each dependant variable. The Generalized Linear Model was also used to analyze each dependant variable with the independent variable treatment and horse.

### Results

The horses differed in plasma cortisol concentrations ( $p < 0.07$ , Table 3.5, Figure 3.1). Horses did not differ in neutrophil count, lymphocyte count, neutrophil-lymphocyte ratios, monocyte counts, eosinophil counts, basophil counts, heart rate, ambient temperature, and horse temperature, regardless of treatment (control vs. Hippotherapy). No variable (cortisol, neutrophil count, lymphocyte count, neutrophil-lymphocyte ratio, monocyte count, eosinophil count, basophil count, ambient temperature, horse temperature, and heart rate) was significant for time or treatment effects alone (Table 3.5).

Cortisol levels were not significantly different across time (Figure 3.2). Heart rate analysis showed a time by treatment interaction (Figure 3.3). Neutrophil count (Figure 3.4), lymphocyte count (Figure 3.5), neutrophil-lymphocyte ratios (Figure 3.6), monocyte count (Figure 3.7), eosinophil count (Figure 3.8), basophil count (Figure 3.9), ambient temperature (Figure 3.10), and horse temperature (Figure 3.10) were not significant for treatment difference across time.

Horse by treatment interaction was significant for cortisol ( $p = 0.1201$ , Table 3.5), lymphocyte count ( $p = 0.0033$ , Table 3.5), neutrophil-lymphocyte ratio ( $p = 0.0015$ , Table 3.5), monocyte count ( $p = 0.0455$ , Table 3.5), basophil count ( $p < .0001$ , Table 3.5), and heart rate ( $p < .0001$ , Table 3.5). This interaction was not significant for neutrophil count, eosinophil count, ambient temperature, and horse temperature.

Heart rate measurements for the mounting ramp showed that Rick and Duncan had the highest heart rate average for all three Hippotherapy sessions. Lilly had the lowest average for heart rate for all three Hippotherapy sessions (Table 3.3).

The horse behavioral survey completed by the head NARHA instructor showed that Rick had desirable traits, but an additional comment stated that he was slightly head shy (Table 3.4). This could possibly account for increased heart rate when his head is worked with. Leonard and Lilly have desirable traits. However, because Lilly had higher cortisol levels and lymphocyte levels for Hippotherapy, but had desirable behavioral traits a recommendation would be to keep her in the program, but constantly monitor her stress levels, and reduce her workload when necessary. Duncan had a few undesirable characteristics, rough, irritable, inconsistent, and restless. Duncan needs to be monitored consistently. Duncan was typically restless and not calm when tied waiting for Hippotherapy sessions to start, however, he behaved appropriately during sessions. Duncan's inconsistency may be due limited experience of one year in Hippotherapy. Duncan may need more frequent training for Hippotherapy. Earl had some undesirable traits, such as rough, irritable, inconsistent, disobedient, and restless. The average rate of desirability for desirable and non-desirable traits was three where one was the most desirable and five was the most undesirable. Earl also had higher cortisol levels and lymphocyte counts during Hippotherapy. He has been removed from the program due to unpredictable behavior. The survey was in agreement with the decision to remove him from the program.

Table 3.1 Age, body weight, body condition score\* (1-9),<sup>62</sup> Hippotherapy experience, and gender for Hippotherapy horses (n = 5)

Horse	Age (years)	Weight (kg)	Body Condition Score (1-9)	Hippotherapy Experience (years)	Gender
Rick	22	527.3	5	7.0	Gelding
Leonard	27	500.0	5	2.5	Gelding
Duncan	13	572.7	6	1.0	Gelding
Lilly	20	563.6	6	1.0	Mare
Earl	8	645.5	8	0.8	Gelding
Average	18	561.8	6	2.4	N/A

\* Henneke<sup>62</sup> Scale 1=extremely thin; 9=extremely fat

Table 3.2 Timeline for measurement parameters (heart rate, blood collection, horse temperature, ambient temperature) for Hippotherapy and control treatments for Hippotherapy horses (n = 5)

Control		Hippotherapy	
1200	Baseline measurements Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature	1200	Baseline measurements Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature
1300	Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature	1300	Session one Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature
1400	Heart rate measurements (20 measurements/hr) Blood collection Horse temperature Ambient temperature	1400	Session two Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature
1500	Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature	1500	Session three Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature
1600	Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature	1600	Baseline measurements Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature
1600	Baseline measurement Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature	1700	Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature
1700	Heart rate (20 measurements/hr) Blood collection Horse temperature Ambient temperature		

Table 3.3 Heart rate (beats per minute) at mounting ramp for all three consecutive Hippotherapy sessions for five horses.

Horse	Ramp HR Session 1	Ramp HR Session 2	Ramp HR Session 3	Average
Rick	67	46	46	53
Leonard	39	36	43	39
Duncan	63*	33	43	46
Lilly	26	28	28	27
Earl	33	33	44	36
Average	46	35	41	40

\*Observable agitated behavior

Table 3.4 Behavioral survey\* completed by the head NARHA instructor for five Hippotherapy horses. Scale: 1 being the most desirable, 5 being the least desirable.

Personality Trait	Rick	Leonard	Duncan	Lilly	Earl
Serious vs. Playful	Serious, 2	Serious, 2	Playful, 3	Serious, 2	Playful
Outgoing vs. Shy	Shy, 3	Shy, 3	Outgoing, 2	Outgoing, 2	Outgoing, 3
Calm vs. rough	Calm, 2	Calm, 1	Rough, 3	Calm, 1	Rough, 3
Seeks vs. avoid	Avoids, 3	Avoids, 4	Seeks, 2	Seeks, 2	Seeks, 3
Submissive vs. dominant	Dominant, 3	Dominant, 3	Submissive, 3	Submissive, 2	Submissive, 3
Easygoing vs. irritable	Easygoing, 2	Easygoing, 3	Irritable, 4	Easygoing, 1	Irritable, 4
Dependable vs. undependable	Dependable, 2	Dependable, 1	Dependable, 4	Dependable, 1	Dependable, 1
Cooperative vs. Uncooperative	Cooperative, 2	Cooperative, 1	Cooperative, 5	Cooperative, 1	Cooperative, 5
Hard vs. easy to spook	Hard to spook, 3	Hard to spook, 1	Easy to spook, 1	Hard to spook, 1	Hard to spook, 3
Approaches vs. avoids	Avoids, 4	Avoids, 4	Approaches, 2	Approaches, 2	Approaches, 3
Sociable vs. loner	Sociable, 3	Loner, 2	Sociable, 4	Sociable, 3	Loner, 3
Consistent vs. inconsistent	Consistent, 2	Consistent, 1	Inconsistent, 3	Consistent, 1	Inconsistent, 2
Friendly vs. combative	Friendly, 3	Friendly, 3	Friendly, 3	Friendly, 3	Friendly, 3
Additional comments	Slightly head shy				

\* Adapted from Anderson, et al, 1999.<sup>31</sup>

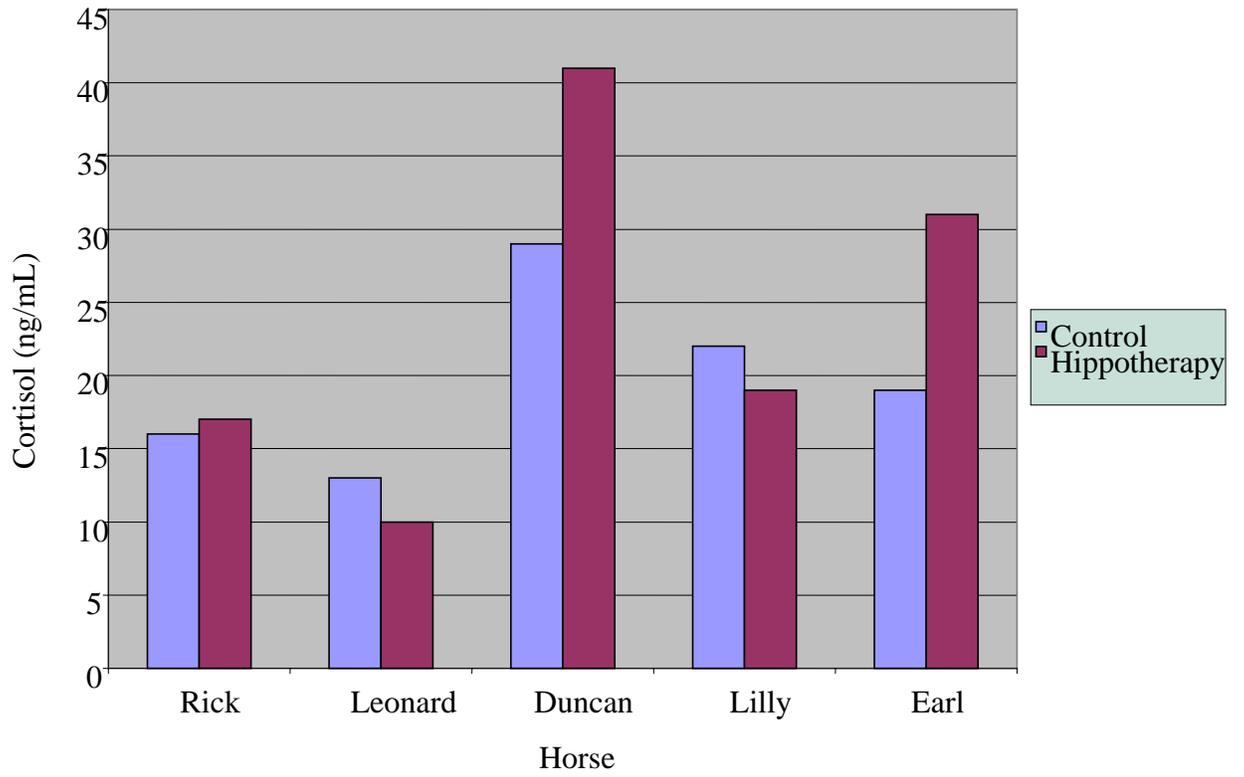


Figure 3.1 Individual cortisol profiles for horses (n = 5) for both control and Hippotherapy treatments.  
P-value 0.

Table 3.5 Control and Hippotherapy LS-mean, standard error<sub>pooled</sub>, treatment p-value, horse p-value, treatment by horse interaction p-value for cortisol, neutrophil count, lymphocyte count, neutrophil:lymphocyte ratio, monocyte count, eosinophil count, basophil count, heart rate, horse temperature, and ambient temperature.

Measure	Control Mean n = 5	Hippotherapy Mean n = 5	SE <sub>pooled</sub>	Trt p-value	Horse p-value	Trt x Horse p-value
Cortisol (ng/mL)	20.57	24.06	2.48	0.37	0.07	0.1201
Neutrophil Count*	34.89	31.93	2.95	0.56	0.33	0.0077
Lymphocyte Count*	62.13	65.16	2.92	0.53	0.29	0.0033
Neutrophil: Lymphocyte Ratio	0.61	0.51	0.08	0.47	0.45	0.0015
Monocyte Count*	0.44	0.56	0.23	0.81	0.71	0.0455
Eosinophil Count*	1.00	1.10	0.28	0.82	0.07	0.2860
Basophil Count*	1.31	0.16	0.87	0.41	0.55	<.0001
Ambient Temperature °Celsius	17.0	20.0	2.16	0.32	0.28	<.0001
Horse Temperature °Celsius	36.7	36.8	0.24	0.72	0.64	<.0001
Heart rate (beats per minute)	34.5	42.5	1.55	0.05	0.05	<.0001

\* All cell counts are out of a total 100 cells.

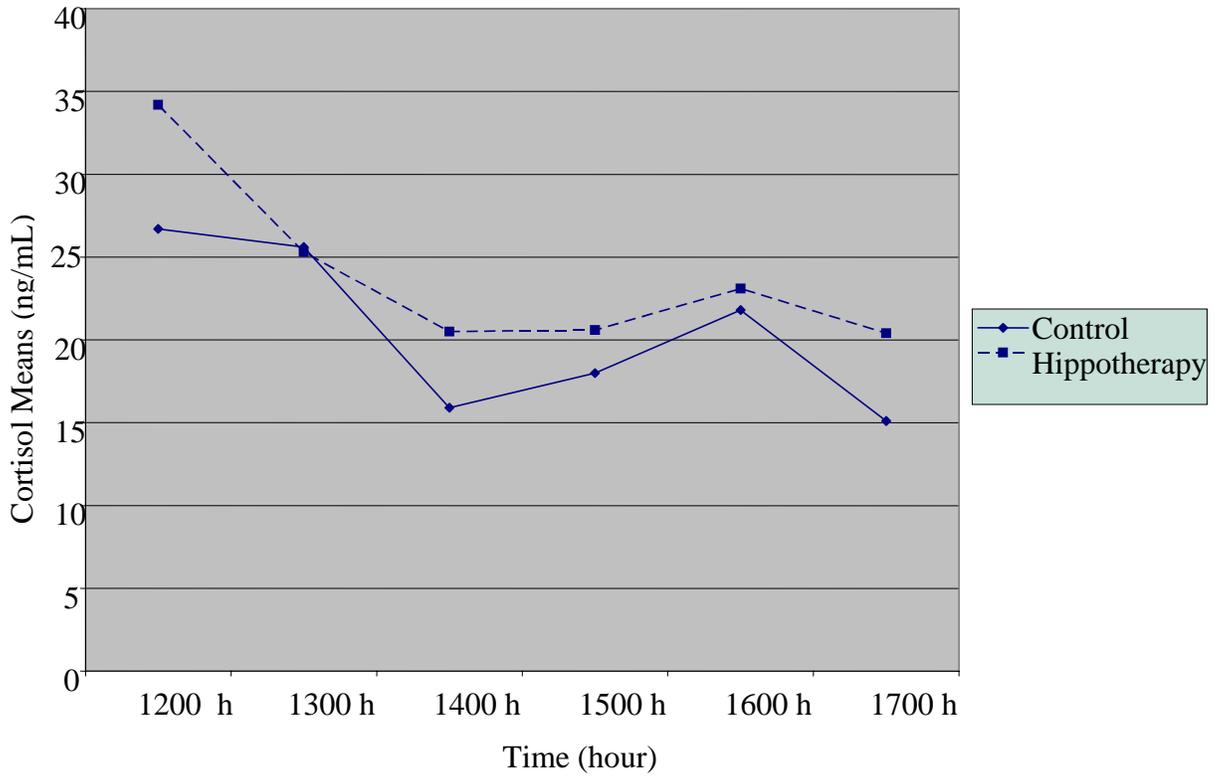


Figure 3.2 Cortisol (ng/mL) means across time (1200 h to 1700 h) for control (n = 5) and Hippotherapy treatments (n = 5). Treatment across time was not significant for cortisol levels.  
 $p < 0.0005$

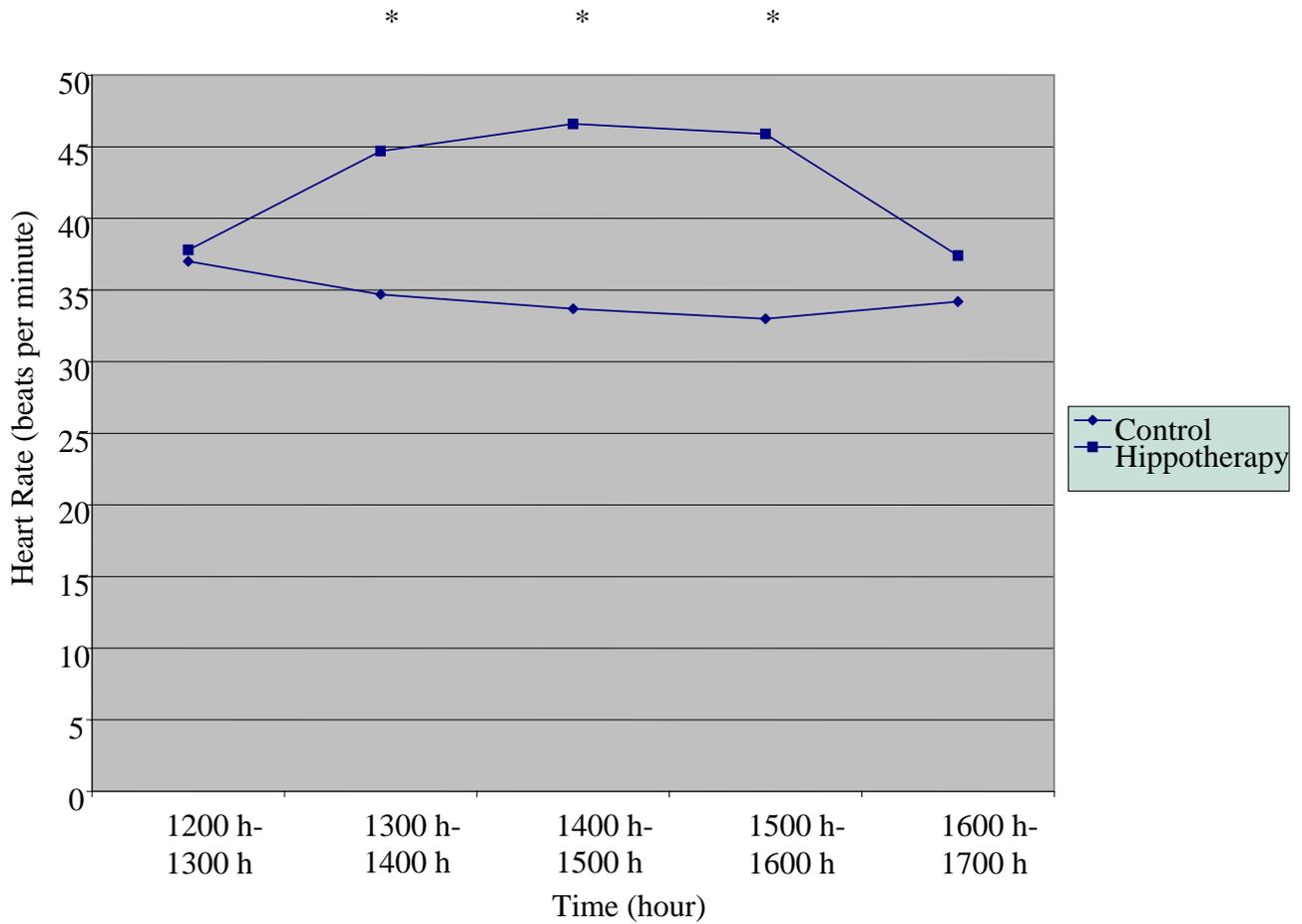


Figure 3.3 Heart rate means for all horses (n = 5/hr. interval) over five time periods (1200 h to 1700 h, 20 heart rate measurement per hour, 100 measurements total for 5 hours) for both control and Hippotherapy treatments.

( $SE_{\text{pooled}}=2.48$ ).

\* indicates  $p < 0.0001$

## CHAPTER IV

### DISCUSSION

Cortisol varied due to time of day; however this effect was not statistically different. Marc and others<sup>42</sup> and Stull and Rodiek<sup>43</sup> reported horses showed a circadian cycle in plasma cortisol. The circadian cycle can be affected by exercise, as levels can increase due to exercise.<sup>40</sup> Cortisol levels were not significantly among treatments due to exercise. Plasma cortisol levels varied among the horses during Hippotherapy. Individual horse temperament and individual personality as demonstrated by Kaiser and others<sup>33</sup> and McCann and others (1998) may explain why some horses had elevated cortisol levels when used in Hippotherapy. Rick and Duncan had a decrease in cortisol levels during Hippotherapy compared to their levels at rest. Lilly and Earl had an increase in cortisol levels during Hippotherapy compared to the Control, levels of plasma cortisol on Leonard were not different regardless of treatment. Rick was consistently used with more disabled Hippotherapy clients. In addition, Rick exercised less as compared to other horses due to the level of children. He stopped many times as directed by the therapists and trotting was avoided. Rick also has been with the UTRC longer than any other horses in the current study. Duncan consistently paced and moved during the control treatment, however, this horse was calm and behaved appropriately during Hippotherapy sessions. Earl has been removed from the Hippotherapy program due to biting behavior. This horse also had elevated levels of cortisol suggesting he experienced stress during Hippotherapy. Another factor for individual cortisol differences in horses is age. Three of the five horses

were twenty years or older. This factor may account for the increase in cortisol levels seen overall.<sup>46</sup>

Horse by treatment interaction was significant for neutrophil counts. This may be explained by examining each horse. Rick, Leonard, and Duncan increased in neutrophil count while in Hippotherapy compared to control. As discussed earlier, neutrophil increase can be transiently effected by exercise.<sup>53</sup> These horses also had a decrease in cortisol while in Hippotherapy as compared to control, as discussed earlier. These two factors, neutrophil count and cortisol level indicate that these three horses were not as stressed during Hippotherapy compared with Lilly and Earl. Lilly and Earl both had increased cortisol plasma, and a decrease in neutrophil count.

Horse by treatment interaction was significant for lymphocyte count. Treatment difference of this interaction can be explained by the fact that lymphocyte counts increased after exercise. McKeever<sup>59</sup> attributed increased lymphocyte counts after exercise to physiological age alterations. The horse by treatment interaction can be explained for all five of the horses due to the averages of lymphocyte counts were on the high end of the normal range during exercise.<sup>55</sup> Time of day was important when looking at the Hippotherapy treatment, where the first and fifth hour were establishing baseline levels, and the second, third, and fourth hours were during exercise. Lymphocyte counts increased for four horses (Rick, Leonard, Duncan, and Earl) while in Hippotherapy as compared to control. However, Lilly had a decrease in lymphocyte counts for Hippotherapy as compared to the control period.

The neutrophil-lymphocyte ratio was significant for horse by treatment interaction. There were no significant effects for treatment, horse difference, or time. This ratio was decreased during Hippotherapy for Rick, Leonard, and Duncan. However, this ratio increased during Hippotherapy for Lilly and Earl. The increase in neutrophil-lymphocyte ratio coupled with increased cortisol levels seen during Hippotherapy for Lilly and Earl indicated stress.<sup>53</sup>

Monocyte count was significant for the horse by treatment interaction. Rick, Leonard, Lilly, and Earl had increased monocyte counts during Hippotherapy compared to control. This is in agreement with Passantino and others.<sup>57</sup> However, Duncan had a decrease in count, though slight. Because 1200 h and 1700 h during the Hippotherapy treatment was sedentary to establish basal levels, this may explain differences in counts seen during Hippotherapy. At 1200 h and 1700 h counts were lower than during Hippotherapy sessions.

Time, horse difference, treatment, and horse by treatment interaction were not significant for eosinophil count. Duncan and Lilly had an increased count for Hippotherapy compared to control. Rick, Leonard, and Earl had a decreased count for Hippotherapy compared to control. All counts were in the normal range according to Tvedten and Korcal<sup>55</sup> except for Rick during the control treatment with a count of six. High eosinophil counts can be due to parasitic infection,<sup>61</sup> although in this trial all horses were on a comprehensive six way anthelmintic rotation.

Basophil count was significant for horse by treatment interaction. Rick, Leonard, and Earl had a decreased basophil count during Hippotherapy compared to the control

treatment. Duncan and Lilly had an increased count for Hippotherapy. All counts were in the normal ranges according to Tvedten and Korcal<sup>55</sup> except Rick in the control treatment with a count of six out of a total of 100 cells. This possibly could be due to allergies.

Heart rate was significant for horse by treatment interaction and time by treatment interaction. All five horses increased heart rate during Hippotherapy compared to control treatments. It should be noted that all heart rates were relatively low and well within the aerobic levels. This can be explained by increased heart rate for exercise.<sup>48</sup> Leonard had the lowest heart rate during Hippotherapy. This is probably due again to more involved disabled Hippotherapy clients were typically assigned to him. Earl received less involved patients. This was due to less experience as a therapy horse and behavioral problems. Rick had the highest heart rate average during Hippotherapy. This possibly could be due to higher respiration rate as seen visibly when evaluating Hippotherapy sessions. For control, Rick had the lowest heart rate average, and Duncan had the highest average. Duncan often paced, pawed, and whinnied which may account for increased heart rate. Heart rate increases during both stress and exercise<sup>48</sup> and this was observed in the current study. Rick, Leonard, and Lilly were the oldest horses. Age factors such as fatiguing faster, lower exercise capacity, increased work load at submaximal heart rate, and decline in anaerobic capacity than their younger counterparts, Duncan and Earl.<sup>41, 49, 59</sup>

The heart rates at the mounting ramp for all Hippotherapy sessions for Rick averaged 53 beats per minute. Leonard's average heart rate for all Hippotherapy sessions was 39 beats per minute. Duncan's average heart rate for all Hippotherapy sessions was 46 beats per minute. Lilly had the most consistent heart rates for all three sessions, with

an average of 27. Earl had an average of 36 beats per minute for all Hippotherapy sessions. Rick had the highest average, Lilly had the lowest average for all three sessions. Session one of hippotherapy for all five horses averaged to 40 beats per minutes. Session two for all five horses averaged 35 beats per minute. Session three heart rates averaged 41 beats per minute. The total average of each session average was 40 beats per minute. Overall heart rates did not change fluctuations drastically for the mounting ramp. This is important because many believe the ramp is a stressful situation for the horse (Table 3.3).

Ambient temperature was not significant for time difference, horse difference, treatment, and horse by treatment interaction. Ambient temperature for the control treatments ranged from approximately 7.7 to 21.6 degrees Celsius. Ambient temperatures for Hippotherapy treatments ranged from approximately 15 to 25.5 degrees Celsius. Temperature was influenced by time of day because temperatures rose consistently in the afternoon after 1200 h, and decreased at 1700 h due to the sun going down for the evening.

Horse temperature was not significant for time, horse, treatment, and horse by treatment interaction. Temperature for control ranged from 36.2 to 37.5 degrees Celsius. Rick and Leonard had consistently lower temperatures during the control treatment as compared to the other horses (Duncan, Lilly, Earl). For Hippotherapy, the temperature range for horses was between 97.3 to 99.6 degrees Fahrenheit. This range of temperature was small. Rick, Leonard, and Duncan had increased temperature during Hippotherapy compared to control temperatures. This increase was due to exercise. However, Lilly and Earl had decreased temperatures for Hippotherapy compared to control temperatures.

Lilly was consistently used with more highly involved Hippotherapy clients that required only walking and many stops as directed by the therapists.

The behavioral survey showed that all of the horses had desirable traits; however, Earl had the most undesirable traits compared to the other horses. His undesirable traits included being rough, irritable, inconsistent, disobedient, and restless can be visible indicators of stress. Duncan and Earl scored inconsistent, only Earl had higher cortisol levels. These behavioral trait comparisons were also seen in Anderson and others.<sup>31</sup> The authors did note, however, that agreement between more than one NARHA instructor on horse behavior was poor. They also stated that horses can possibly behave differently for different instructors. The horses with undesirable traits typically had higher cortisol levels, also in agreement with Anderson and others.<sup>31</sup> Behavior surveys can assist therapy programs in assessing suitability in relation to stress-related behaviors.

### Conclusions and Implications

Two of the five horses, Lilly and Earl had increased cortisol levels, increased neutrophil-lymphocyte ratios, and increased monocyte counts that indicated they experienced stress during Hippotherapy. Behavior and individual personality may have accounted for individual differences of stress responses seen in therapy horses. Stress measurements coupled with behavioral evaluations from NARHA instructors are a management practice that can help therapeutic riding centers better select potential therapy horses and assess current therapy horses to prevent stress and burnout. The recommendation from NARHA that therapy horses should not work more than three consecutive sessions may need to be changed for less experienced horses to less than

three consecutive sessions. For seasoned therapy horses, this recommendation does not need to be altered. However, behavior needs to be continually assessed in seasoned therapy horses to prevent burnout. Cortisol, heart rate, and white blood cell differentials provide a means to evaluate if individual horses are suitable for Hippotherapy. Even though Cortisol coupled with cell counts indicates stress, this may not be practical for all therapeutic riding centers to conduct. However, most centers could collect blood, have whole blood slides created, and cells counted to help indicate if horses are under stress. Further research is warranted to increase the numbers with the purpose of making recommendations to monitor horses used in equine-assisted activities.

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APPENDIX

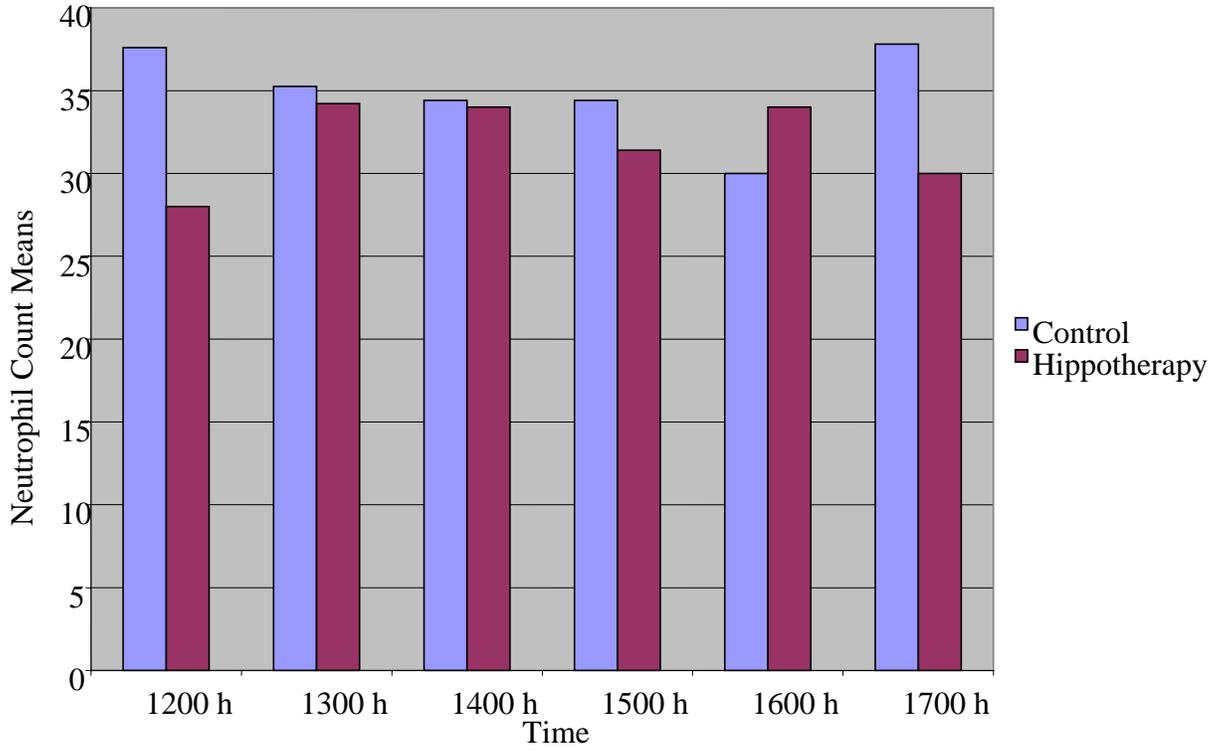


Figure 3.4 Neutrophil count means for both treatments (control and Hippotherapy) over six time periods (1200 h to 1700 h) for all horses (n = 5).

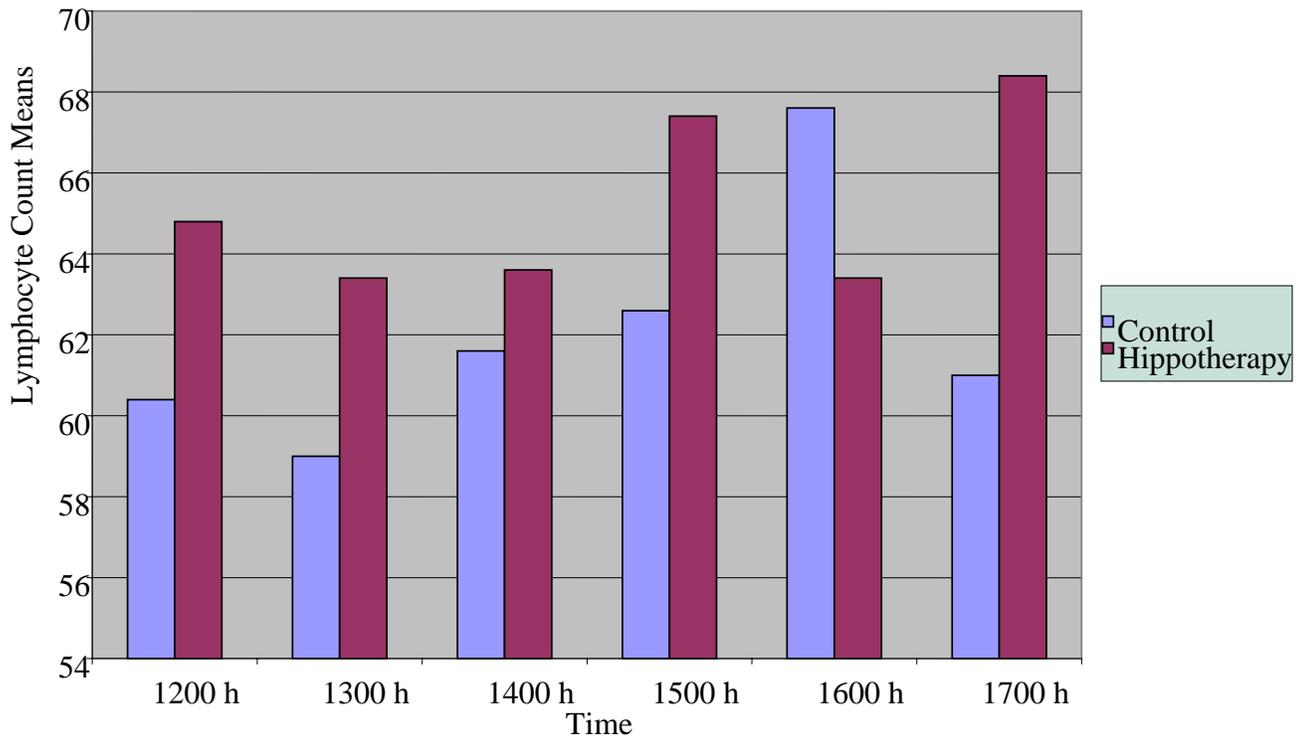


Figure 3.5 Lymphocyte count means for control and Hippotherapy treatments over six time periods (1200 h to 1700 h) for all therapy horses (n = 5).

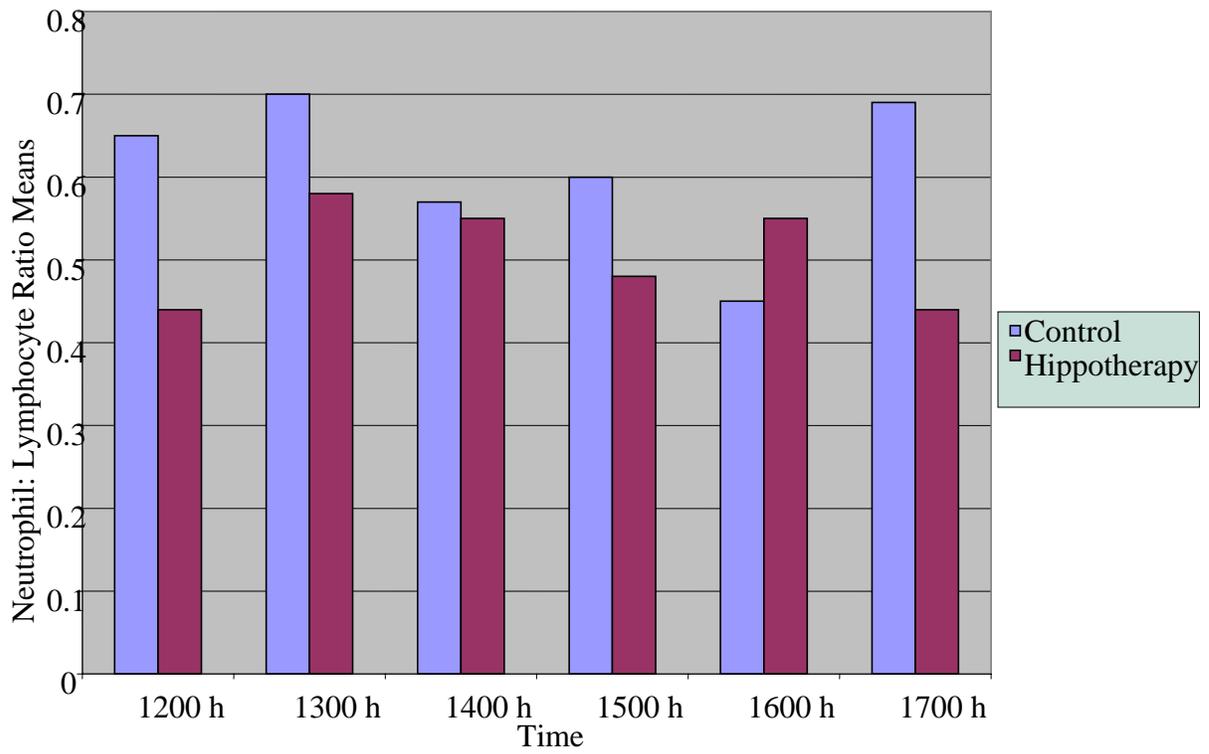


Figure 3.6 Neutrophil-lymphocyte ratio means over six time periods (1200 h to 1700 h) for control treatment and Hippotherapy treatment for five horses.

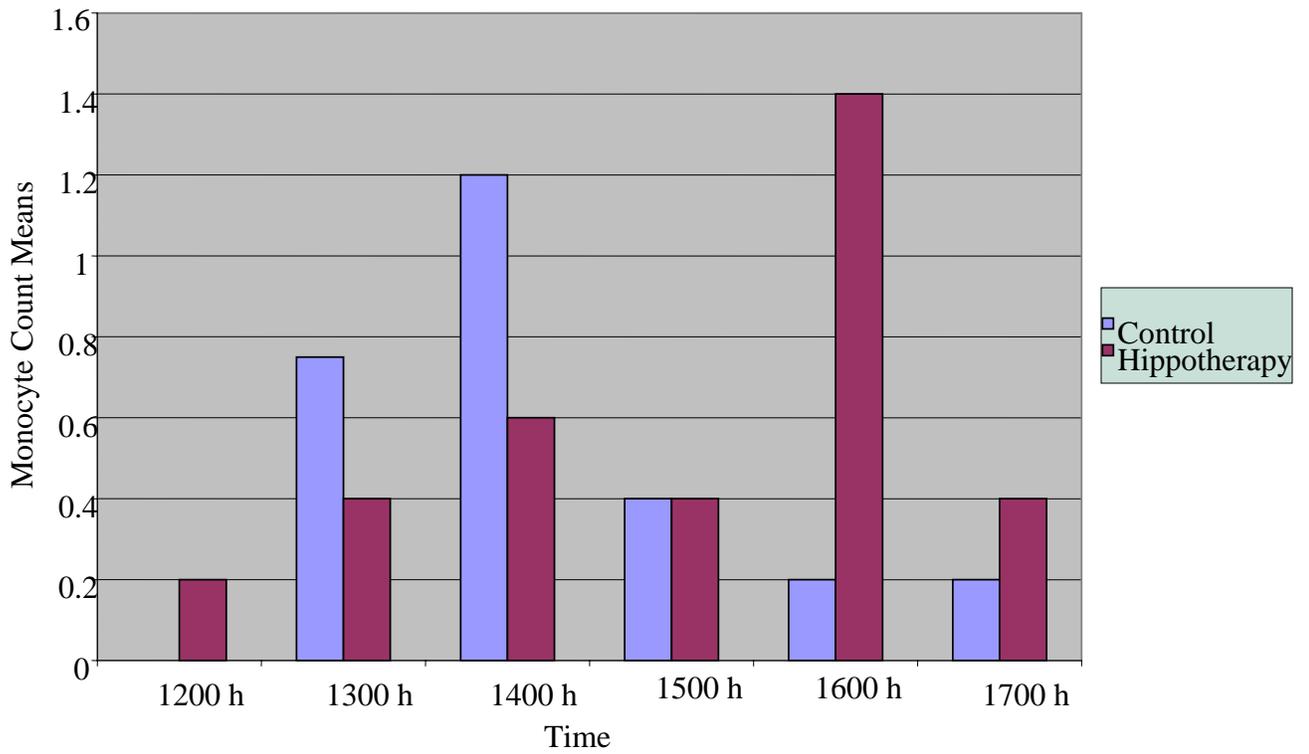


Figure 3.7 Monocyte count means over six time periods (1200 h to 1700 h) for control and Hippotherapy treatments for five horses.

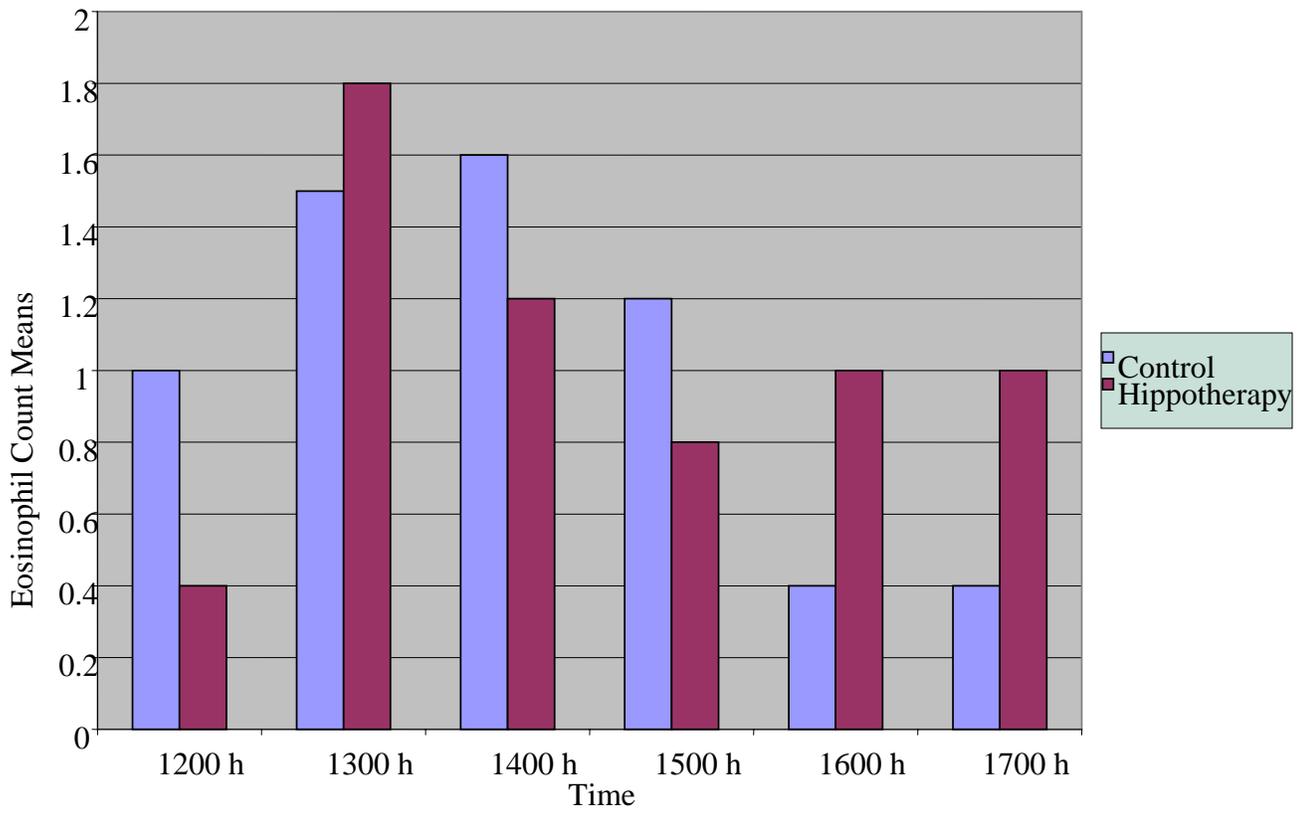


Figure 3.8 Eosinophil count means for control and Hippotherapy over six time periods (1200 h to 1700 h) for all horses (n = 5).

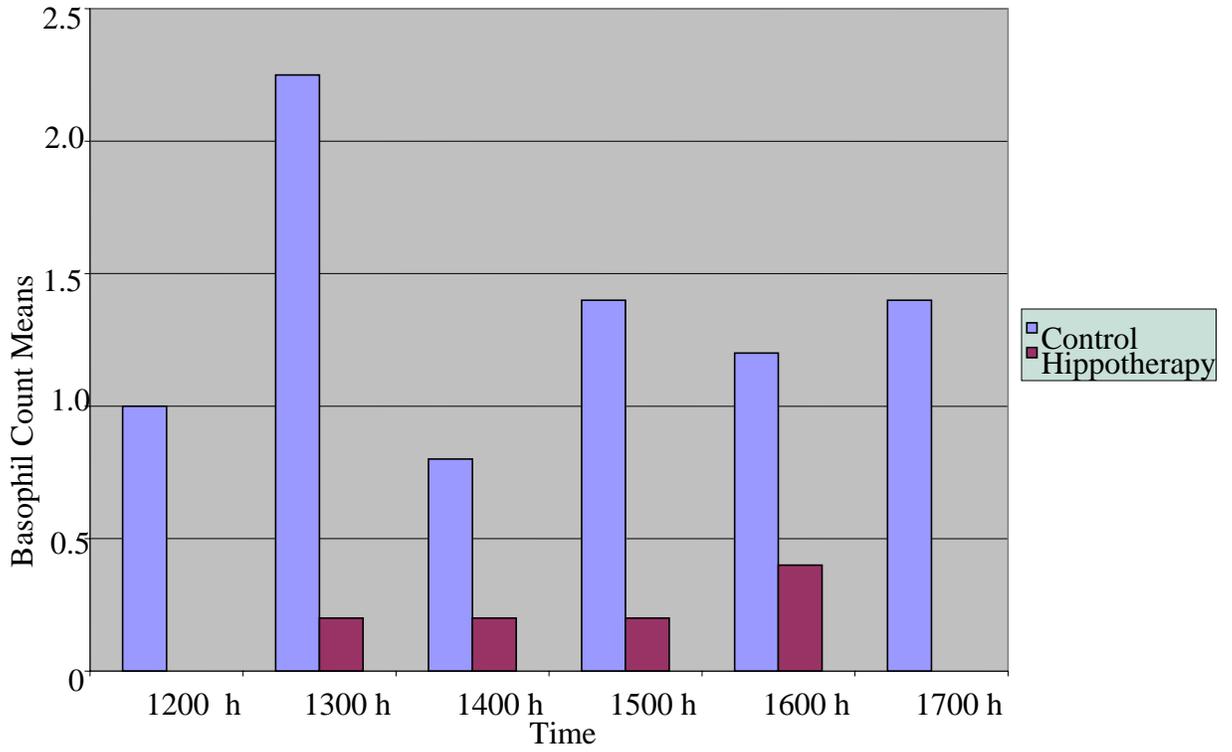


Figure 3.9 Basophil count means for both treatments (control and Hippotherapy) for six time periods (1200 h to 1700 h) for five horses.



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