BLOOD LACTATE AND HEART RATE RESPONSE OF YEARLING HORSES ON A PROGRESSIVE WORKLOAD

An Undergraduate Research Scholars Thesis

by

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RESEARCH COMPLIANCE CERTIFICATION

Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Merina Jackson, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisor prior to the collection of any data used in this final thesis submission.

This project required approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Blood Lactate and Cardiovascular Response of Yearling Horses on a Progressive Workload

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Blood lactate concentrations and heart rate levels surrounding exercise provide valuable insight into the physiological response to advancing workloads, but minimal research has been published involving yearling horses. Therefore, the objective of the study was to determine blood lactate and heart rate response to increasing exercise intensity in yearling horses. It was hypothesized that blood lactate levels would peak immediately following exercise, and gradually decrease over a 3 h post-exercise period. Comparatively, heart rate levels would increase over time in response to an increasing exercise intensity. To test this, 32 Quarter horses of similar background (480-520 d of age; 16 geldings and 16 fillies) were randomly assigned to one of four groups (n=8/group) and exercised using an 8-panel free-stall walker. Exercise bouts ranged between 30-45 min and were divided equally in the clockwise and counterclockwise directions. Each wk, 4 horses per group were randomly assigned and equipped with onboard heart rate monitors during exercise each d throughout the course of this study. Blood lactate measurements were taken at the beginning of an increase in exercise intensity, noted as Test 1, Test 2, and Test 3. Test 1 consisted of walking at 1.75m/s, Test 2 consisted of horses alternating between the

walk and trot at 1.75 and 4.0 m/s and Test 3 consisted of horses alternating between the walk, trot, and canter at 1.75, 4.0. and 6.0 m/s respectively. Blood samples were obtained at the onset (PRE) and immediately (H0), half (H0.5), one (H1), and three (H3) h post-exercise. Heart rate levels were continuously monitored throughout the course of exercise. Mean blood lactate was determined using two portable lactate analyzers previously validated for use in horses. Data were analyzed using PROC MIXED of SAS with PRE-blood lactate values used as a covariate, and no covariate was used for heart rate data. Heart rate increased over time (P <0.01) in response to an increasing exercise intensity. There was a group × test × time interaction (P<0.01), where Group 3 reached peak lactate concentration at H0 during Test 2, while all other groups increased blood lactate concentrations over time to H3 post. Mean lactate (1.07 ± .08 mmol/l) post-exercise indicates that horses were able to clear lactate without a sufficient increase in systemic distribution. During Test 2, mean lactate for Group 3 peaked at H0 (1.23 ± .08 mmol/l) with a gradual decrease during recovery. Although there was an increase in heart rate levels over the course of the study, mean heart rate remained within sub-maximal values. Lactate concentrations varied across all three test, but all values remained below the anaerobic threshold, similar to mature horses, which suggests that sub-maximal exercise in young horses does not elicit an anaerobic response and lactate accumulation.

DEDICATION

To my mom, thank you for being my best friend and supporting my endlessly.

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Contributors

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The materials used for blood lactate and heart rate response of yearling horses on a progressive workload were provided by Jessica Leatherwood. The analyses depicted in blood lactate and heart rate response of yearling horses on a progressive workload were conducted in part by Brittany Silvers and these data are unpublished.

All other work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

BW	Body Weight
d	Day
FFA	Free Fatty Acids
g	Gauge
HRmax	Maximum Heart Rate
m	Meters
min	Minute
mL	Milliliters
Mmol/l	Millimoles per Liter
n	Number
TAMU	Texas A&M University
Week	Wk

1. INTRODUCTION

The horse is an exceptional athlete, outperforming almost every other animal similar in size. Horses have the ability of jumping immense heights, running at incredible speeds, and multiple other activities dependent on their discipline. However, if proper training is not preformed, loss of optimal performance and injuries may occur (Logan et al., 2021). All equestrian disciplines require an intense training program to reach peak performance, but one of the most notorious equine trainings is preparing young horses for sale. Early exercise in the juvenile horse has a positive effect on the physiological system and primes the horse for an athletic career (Rogers et al., 2012). The equine industry demands that there is an introduction to early exercise and training to achieve peak performance condition by 2-3 years of age. These young horses have an average of 12-13 wks of intense training to ensure they are ready for sales and competition (Bowell et al., 2012). The effectiveness of training programs can be evaluated using different physiological parameters, including heart rate levels and blood lactate, to ensure optimal performance of the horse is being met (Hinchcliff et al., 2008).

Heart rate and blood lactate measurements are the bases of an exercise test for the athletic horse (Hinchcliff et al., 2008). Heart rate measurements evaluating the horses' exercise capacity are commonly used in determining the cardiovascular adaptation to a training program. As heart rate increases, blood lactate concentrations are expected to increasing proportionally (Kang et al., 2017). The cardiovascular system is responsible for delivering blood to the muscles, and removing byproducts of energy metabolism, including lactate. Lactate is an acidic byproduct which lowers the pH within the muscle fibers, causing increased muscle fatigue. Recovery from muscle fatigue takes about 3 hours for lactate to be completely removed from the bloodstream (Clayton, 1991).

Minimal longitudinal studies of blood lactate and heart rate levels involving the yearling horses have been conducted in the research field but these measurements following exercise provides valuable insight into the metabolic response to different workloads. Therefore, the objective of this study was to evaluate blood lactate concentration and heart rate levels in response to increasing exercise intensity in yearling horses. It is predicted that heart rate levels will peak during exercise, and gradually increase over the course of the 168-d study. Blood lactate concentrations are expected to peak immediately following exercise, and gradually decrease over a 3-h post exercise period.

2. REVIEW OF LITERATURE

2.1 Exercise and Training of the Young Horse

Exercise is essential to the strengthening and maturation of the working horse and is an imperative component of sales preparation and early performance training in horses of all breeds and disciplines. Exercise is a common term used that refers to forced movement or activity. Comparatively, training is defined as a long-term process of repeated bouts of exercise, resulting in increased fitness (Marlin et al., 2006). Training programs are designed to help increase a horses' exercise capacity, increase the time to onset of fatigue, and improve overall performance. Different exercise protocols, including both submaximal and maximal exercise, are compiled to create a training program. Equine training is a vital component when preparing young horses for sales preparation, and often involves utilizing submaximal and maximal exercise to create an effective training protocol (Hinchcliff et al., 2008).

2.1.1 Submaximal Exercise

Submaximal exercise represents 50-75% of the maximum heart rate during high intensity exercise and has been determined to average between 110-160 beats/minute (Hinchcliff et al., 2008). Submaximal exercise is accompanied by aerobic metabolism, which uses energy derived from the horses' body, via the citric acid cycle and oxidative phosphorylation (Pagan, 1998). In equine disciplines that require stamina, including preparing young horses for sales, submaximal exercise relies predominately on oxidative phosphorylation to contribute to the replenishment of ATP during exercise (Votion et al., 2012).

2.1.2 Maximal Exercise

Maximal exercise is defined as exercise which peaks heart rate levels to 70-80% of the maximum heart rate. Maximum heart rate is independent to all horses and can be determined by a plateau in the heart rate response averaged to be between 220-240 beats/min. (Marlin et al., 2006). Maximal exercise is supported by anaerobic glucose metabolism, which breaks down glucose into lactic acid (Pagan, 1998).

2.2 Blood Lactate

Lactate is a toxic waste product of anaerobic glucose metabolism, or glycolysis, which accumulates in the muscle and blood during high intensity exercise (Clayton, 1991). Carbohydrates and ADP are synthesized to anaerobically produce ATP and lactate in this reaction. While most lactate is removed from the muscles by the bloodstream, some can be used as an energy substrate to support aerobic metabolism during submaximal exercise. During high intensity exercise, lactate is produced at a much faster rate than it can be removed by the bloodstream, creating a buildup within muscle fibers and blood. Lactate is acidic, and lowers the pH of muscle fibers, resulting in fatigue. Horses average a three-hour post exercise period for lactate to be completely removed by the bloodstream (Clayton, 1991). Lactate is dependent on the rate of lactate production in muscles, diffusion of lactate in the muscles to the blood, and the rate at which it is removed (Clayton, 1991). Blood lactate concentration provides valuable insight into the anaerobic response to increasing exercise intensity in yearling horses and can be used as a quantitative method of determining the level of exercise fitness in the horse (Kang et al., 2017).

2.2.1 Anaerobic Threshold

The anaerobic threshold, or the onset of blood lactate accumulation is defined as the intensity of exercise at which the blood lactate concentrations rise above 4mmol/L. V_{LA4} is determined to be the velocity at which a horse breaks the anaerobic threshold (Rainger, 1994). The anaerobic threshold is an important indicator that a workload is requiring the horse to produce a significant amount of energy, and is a vital factor used when training for any equine discipline. V_{LA4} is unique to every horse, and can be determined by collecting blood lactate following multiple different workloads. While V_{LA4} is independent to each horse, research has indicated that unfit horses cross the anaerobic threshold at speeds of 400 m/min, and fit horses at 500 m/min (Clayton, 1991).

3. MATERIALS AND METHODS

3.1 Horses and Treatments

Thirty-two yearling Quarter horses (16 geldings and 16 fillies) all of similar background were randomly assigned to one of four groups during the course of this 168-d trial. Prior to the start of this study, horses were determined to be free of lameness and other abnormalities at the start of this study by a board-certified veterinarian (Texas A&M Large Animal Hospital, College Station, TX). Once enrolled in the study, horses were housed in individual stalls with attached runs (3.6 m × 10.8 m) at all times during the course of the project, except for designated exercise times, to control physical activity and mimic industry rearing programs. Horses were allowed *ad-libitum* access to coastal bermudagrass (*Cynodon dactylon*) hay and water. Horses were fed 1% BW (as-fed) of a commercially available concentrate (ProElite ® Growth, Cargill, Elk River, MN) that was weighed using calibrated feed scales and offered twice daily, individually at 0600 and 1800 h. Horses were weighed every 21 d with intake adjusted accordingly. Diets were formulated to meet or slightly exceed nutrient requirements for yearling horses on a moderate workload undergoing rapid growth (NRC, 2007). All horses received standard preventative healthcare which included deworming and hoof trimming at regular intervals throughout the study. Lameness evaluations were conducted every 6 wk, and any horse exhibiting lame was removed from the study until further evaluation at the discretion of a licensed veterinarian associated with the project. Exercise Protocol

Exercise protocol for the current study was divided into two phases and designed to mimic preparing yearling horses for sales. Horses were randomly assigned to one of four groups to allow for consistent exercise 5 d/wk on an 8 panel-free stall walker (Priefert; Mt. Pleasant, TX). D 1-7 consisted of horses exercising 25 min/d for 5 d/wk at the walk (1.75 m/s), divided equally in the clockwise and counterclockwise direction. Walking exercise increased 5 min per wk until 45 min of maximum walking was achieved on d 35. Once maximum walking exercise was accomplished on d 35, 5 min of the trot (4.0 m/s) was implemented in both the clockwise and counterclockwise direction. Exercise was continued 5 d/wk for 45 min/d until the end of Phase I (Table 1). Between the end of Phase I and the introduction of Phase II, horses were given 14 d of rest following a biopsy. Phase II (d 99-168) of the study consisted of horses being exercised on the same 8-panel free-stall walker. Ds 99-112 consisted of horses alternating between the walk and trot 30 min/d for 5 d/wk until d 113, when maximum exercise was achieved. Horses maintained the maximum exercise protocol for the duration of the study (Table 2).

***Table 1**: Phase I Exercise Protocol (m/s) for d 1-84 of the study*

Day	Clockwise		Counterclockwise	
1-7	walk* 12.5 min		walk 12.5 min	
8-14	walk 15 min		walk 15 min	
15-21	walk 17.5 min		walk 17.5 min	
22-28	walk 20 min		walk 20 min	
29-35	walk 22.5 min		walk 22.5 min	
36-42	walk 17.5 min	trot** 5 min	walk 17.5 min	trot 5 min
43-49	walk 17.5 min	trot 5 min	walk 17.5 min	trot 5 min
50-56	walk 17.5 min	trot 5 min	walk 17.5 min	trot 5 min
57-63	walk 17.5 min	trot 5 min	walk 17.5 min	trot 5 min
64-70	walk 17.5 min	trot 5 min	walk 17.5 min	trot 5 min
71-77	walk 17.5 min	trot 5 min	walk 17.5 min	trot 5 min
78-84	walk 17.5 min	trot 5 min	walk 17.5 min	trot 5 min

[1] Walk at 1.75 m/sec
[2] Trot at 4.0 m/sec

Table 2: *Phase II Exercise Protocol (m/s) for d 99-168 of the study*

Day	Clockwise	Counterclockwise
99-105	walk[1] 5 min, trot[2] 10 min	walk 5 min, trot 10 min
106-112	walk 5 min, trot 5 min, canter[3] 5 min	walk 5 min, trot 5 min, canter 5 min
113-119	walk 5 min, trot 3 min, canter 7 min	walk 5 min, trot 3 min, canter 7 min
120-168	walk 5 min, canter 20 min	walk 5 min, canter 20 min

[1] Walk at 1.75 m/sec
[2] Trot at 4.0 m/sec
[3] Canter at 6.0 m/s

3.2 Sample Collection

3.2.1 Heart Rate Collection

Horses were randomly assigned to have their individual heart rate per group were randomly assigned to have heart rate collected during exercise over the duration of this study. Polar Equine Heart Rate Sensors (Polar USA, Newark, NJ, v 10) were attached to Polar Equine Sensor Bands (Polar USA, Newark, NJ) and approximately 5mL of Spectra 360 Electrode Gel (Parker Laboratories Inc., Fairfield, NJ) was placed along the pads of the band. Electrode gel was used to allow for a better communication of the electrical impulses from the horses' body and the pads on the sensor band for accurate heart rate collection (Parbrook et al., 1985). Bands were placed on the designated horses at the beginning of each exercise bout to monitor heart rate levels throughout. Mean heart rate values were used to evaluate cardiovascular response to exercise. Heart rate data was recorded via Bluetooth and collected using the KerClockIt App

(Kentucky Equine Research, Versailles, KY). Data were analyzed and weekly maximum and mean heart rate was collected to evaluate change in fitness over time.

3.2.2 Blood Collection

Blood lactate was measured at the beginning of Phase I, Phase II, and when maximum exercise was reached on d 113 of the project (Test 1, 2, and 3, respectively).. On test d, blood was obtained before exercise (PRE), and immediately (H0), half (H.5), one (H1), and three (H3) hours post exercise. Blood was obtained via jugular venipuncture using 20 g × 1 in needles and placed into non-additives sterile tubes (BD Vacutainer, Franklin Lakes, NJ).

3.3 Lactate Analysis

Blood lactate concentrations were analyzed using a portable Lactate Plus Analyzer (Nova Biomedical, Waltham, MA). Using pipettes, blood was obtained from each vacutainer and placed onto a Lactate Plus Meter Test Strip (Nova Biomedical, Waltham, MA) and inserted directly into the analyzer. Blood lactate was analyzed using two different Lactate Plus Analyzers to increase efficiency, and a mean value determined.

3.4 Statistical Analysis

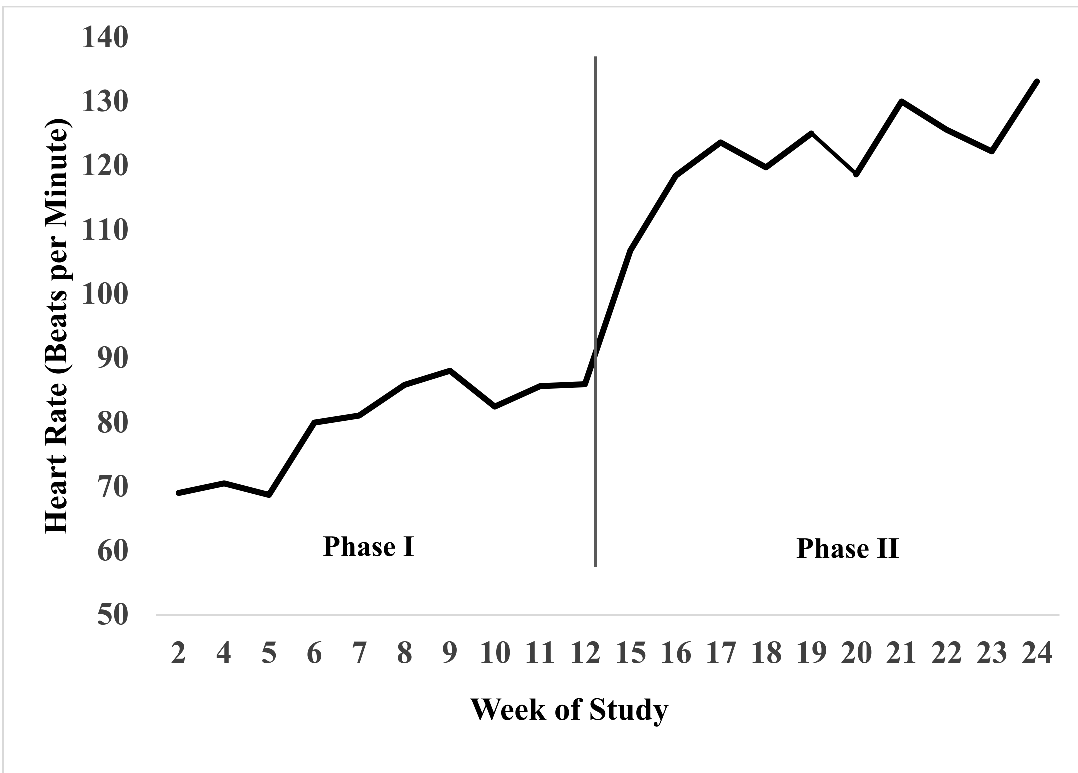
Data were analyzed using the PROC MIXED method of SAS (SAS Inst. Inc., Cary, NC.). Data were tested for normality and outliers were removed. Heart rate data were analyzed with a main effect of time. Lactate data were analyzed with main effects of group, test, time and their respective interactions. Pre-exercise lactate levels were used as a covariate to account for baseline differences in blood lactate concentration. Covariate structure was utilized to account for pre-exercise differences in lactate. Significance was declared at $P \leq 0.05$ and a trend at $P \leq 0.10$.

4. RESULTS

4.1 Heart Rate Levels

Mean heart rate was evaluated over the course of the study on a weekly basis and. heart rate values increased throughout the 168-d project (P <0.01) in response to increasing exercise intensity (Figure 1).

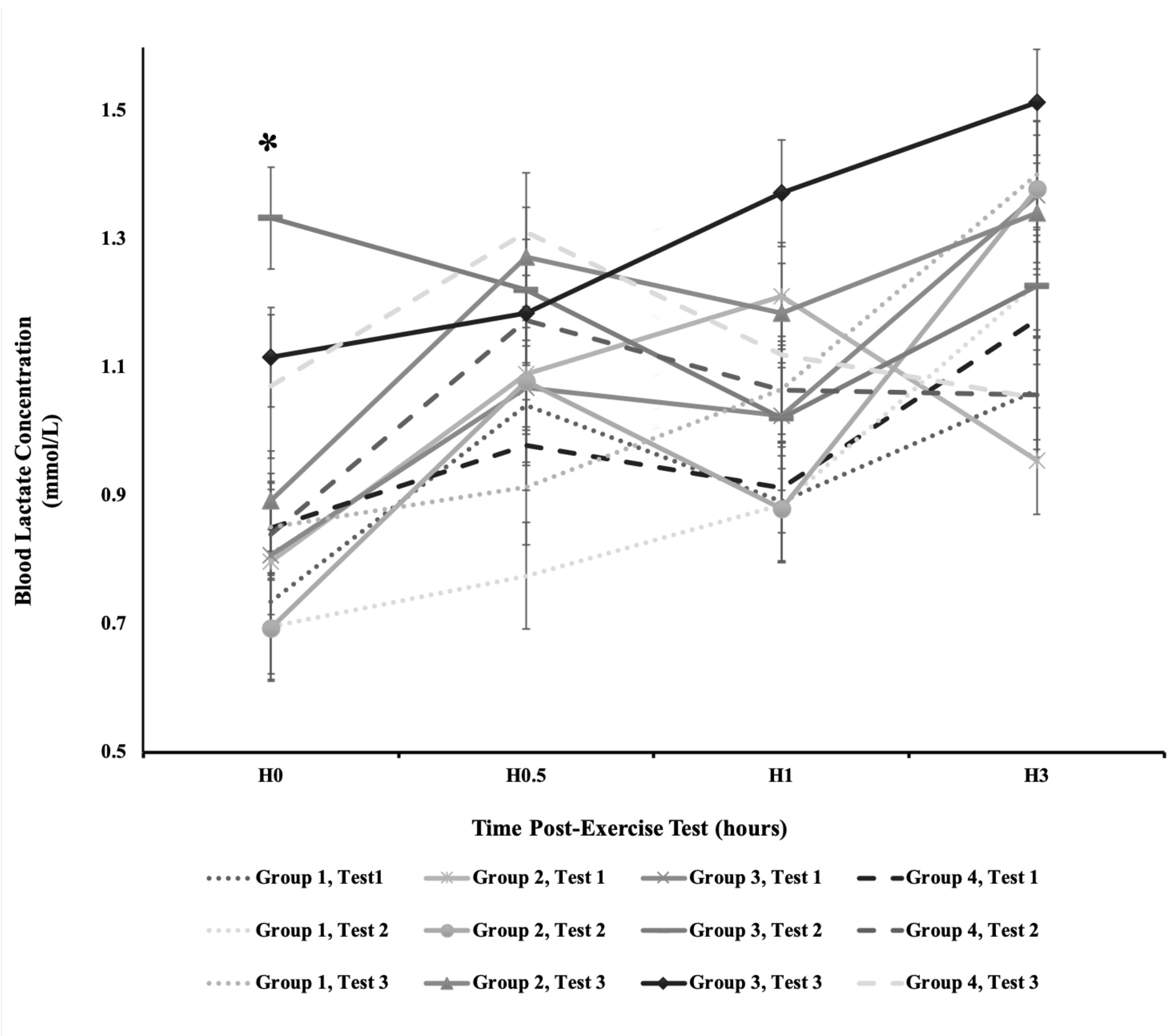
Figure 1: *Weekly mean heart rates during Phase I and Phase II of the study*



4.2 Blood Lactate Concentration

There was a group × test ×time interaction ($P < 0.01$) on blood lactate concentration, where Group 3 reached peak lactate immediately following exercise on Test 2, while all other groups increased blood lactate concentration over time to h three post exercise (Figure 2).

Figure 2: *Blood lactate response over time for Test 1, Test 2, and Test 3*



5. DISCUSSION

This study evaluated the effect of increasing exercise intensity on blood lactate concentrations and heart rate levels in yearling horses. As expected, mean heart rate levels increased in response to an increasing exercise intensity over the course of this 168-d study. Although these values increased, all heart rate levels remained within the submaximal range. Mean heart rates were recorded to be between 70-133 BPM. The highest speed reached was 6.0 m/s during Phase II on ds 113-168, which indicated that HRmax was never met. Although HRmax is independent to each horse, it is estimated to be 70-80% of the maximum heart rate, and attainable at high intensity speeds (Marlin, 2006). It can be suggested that HRmax was not met because exercise speeds were not fast enough (13.3 – 16.7 m/s) to reach maximal heart rate output of the horse and there was no evident plateau in maximum heart rates when interpreting the data (Hodgson, 2014). While HRmax was not met, there was an increase in fitness over time in response to submaximal exercise ($P<0.01$).

Blood lactate surrounding submaximal exercise has been determined to not break the anaerobic threshold, due to V_{LA4} not being high enough in this study. In other research surrounding equine heart rates and blood lactate concentrations following submaximal exercise shows similar findings. A similar study conducted showed mean heart rate values of horses to be 45 beats/min at rest and 139 beats/min following 20 min of exercise at 4 m/s, while mean blood lactate was recorded at 1.1 mmol/l and 1.2 mmol/l immediately following submaximal exercise (Lindner et al., 1998). It can be suggested that submaximal exercise does not elicit an anaerobic response or enough lactate accumulation to break the anaerobic threshold.

Blood lactate measurements showed gradual increase in lactate concentration over time to hour 3 post exercise, except for Group 3, Test 2, who reached peak lactate immediately following exercise. A possible explanation to this interaction could be attributed to the time morning concentrate was consumed in relation to exercise being performed. There are a multitude of different energy sources a horse can utilize, including intramuscular glycogen and triglycerides and extra muscular stores such as adipose tissue and liver glycogen (Pagan, 1998). Multiple factors determine the type of energy used from storage include: speed, duration of work, feed, fitness, and other factors. This indicates that concentrate consumed before exercise may have been the main energy source used during exercise for Group 3, Test 2, explaining why there was a peak in lactate immediately following exercise.

Additional extraneous factors surrounding this study were horses having two wks off from controlled exercise, and the validity of the Polar Equine Heart Rate monitor belts. Horses were allotted two wks off from controlled exercise between Phase I and Phase II for rest and recovery. This posed the possibility of horses losing fitness levels, and presented the issue of higher heart rates and lactate levels when horses were re-exposed to exercise after two wks off. A study conducted involving loss of fitness in equine indicated that increase expected in response to exercise if horses are not exercised for more than 7 d (Clayton, 1991). The Polar Equine heart rate monitor proved to be challenging at times in regards to recording accurate heart rates. Issues arose during exercise where the Bluetooth connection would disconnect and provide inaccurate readings. These data points were removed from the overall data analysis. In order to improve future studies similar to this one, investigation of other equine heart rate monitoring devices may prove beneficial.

6. CONCLUSION

The objective of this study was to evaluate blood lactate and heart rate response to increasing exercise intensity in yearling horses. In conclusion, the results from this study indicate the current submaximal exercise protocol does not elicit an anaerobic response in yearling horses. Mean weekly heart rate values remained within the submaximal range as hypothesized, but blood lactate increased gradually over a 3 h post exercise period, except for Group 3, Test 2, who reached peak lactate immediately following exercise. While lactate results varied, it is important to note that mean lactate (1.07 ± .08 mmol/L) post-exercise suggests that horses were able to clear lactate without a sufficient increase in systemic distribution and did not break the anaerobic threshold. Similarly, the average heart rates on each test d, while varied, all remained below maximal heart rate levels averaging 200 beats/minute, also indicating that the intensity of exercise was not enough to elicit an anaerobic response.

Heart rate values increased in response to an increase in workload throughout the course of the study. Overall, blood lactate measurements remained below the anaerobic threshold, and there was not a notable peak in lactate observed. If the training protocol would have included one more phase of higher intensity exercise with speeds up to 15m/s, the anaerobic lactate threshold and maximal heart rate range may have been met. Further experimentation should include higher exercise speeds to elicit a lactate response, which is directly correlated with HRmax.

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