INFLUENCE OF RESRICTED MOVEMENT ON PHYSICAL FITNESS AND THE WELL-BEING OF PREGNANT MARES

By

CELESTE RENEE SPARKS

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Thesis Approved:

Don R. Topliff
Thesis Adviser

Thomas C. Collins
Dean of the Graduate College

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Chapter I

Introduction

The Pregnant Mare Urine (PMU) farms have recently been under scrutiny by animal right's activists due to extended periods of confinement these mares are subjected. These activists have stated that mares' fitness decreases throughout the urine collection periods on these farms. The well-being of the mares on PMU farms is monitored by company field representatives whom inspect the farms monthly (Jones 1996), and and also by compliance to a code of practice. This Recommended Code of Practice for PMU farms, section 4.6, states, "All horses on a PMU farm should be provided with as much exercise as is necessary for their welfare." To date, there is no data available to define the exercise requirement for horses.

A variety of exercise programs are established at individual PMU farms. Some mares are turned out every 4-5 days, some more frequently, and some mares are not exercised for the whole collection period, approximately 5-6 months. It has been noted that a few mares became agitated when initially removed from their tie stalls and allowed exercise, which indicates that removing these mares from their daily routine may be a source of behavioral stress (Coleman, personal communication).

The North American Equine Ranching Information Council (NAERIC) conducted a survey in 1994 of 387 PMU ranches in Canada and North Dakota (NAERIC 1994). This survey was conducted to determine the extent of exercise programs utilized under current PMU management practices. Results indicated that 97.7% of the PMU farms have established exercise programs in which mares are exercised an average of six days per month. The remaining 2.3 %

either did not respond to the survey or do not have established exercise programs. Seventy six percent of the farms, with exercise regimens, alter their exercise programs to meet the behavioral needs of each horse, and the remaining 21% of the PMU farms indicated established exercise programs with standard levels for the entire herd.

Fitness is defined by Stedman's/Webster's New World Medical Dictionary (1987) as suitability or well-being. These mares that are utilized during urine collection periods are suitable for the PMU farms because they are "fit" to carry their foal to term. The majority of PMU farms exercise their mares during urine collection periods, however there is no defined exercise requirement for horses. The objective of this study was to determine the effects of extended periods of restricted movement on response of mares to aerobic exercise and to begin to establish guidelines for exercise in mares housed under current PMU conditions.

Chapter II

Literature Review

Confinement studies

In a natural environment horses are social creatures, which spend most of their time in contact with like-species. In confinement situations, behavioral changes have been used as an indicator of a horse's well-being. Behavioral responses of mares to short-term confinement and social isolation was researched by Mal et al (1991). Two weeks prior to the initiation of that study, mares were brought in from pasture, weighed to determine ACTH dosage, assigned a temperament score ranging from one to nine (based upon ease of catching), assessed for reaction to surprising stimuli (like sudden movements), and reaction to routine handling procedures (like weighing). Twelve mares were blocked by age and temperament and placed into each of three separate housing conditions. The first housing condition consisted of pasture (4.5 ha) with unlimited contact between horses. The second housing condition consisted of confinement in 4.2 m x 6.0 m stalls. These stalls allowed auditory, visual, and tactile contact between mares. The third housing condition consisted of an isolation stall (3.6 m x 3.6 m). This isolation stall allowed no tactile, auditory or visual contact between horses, and created an environment with reduced sensory stimuli.

After the mares were in their designated housing condition for 48 hours, data was collected for an hour, and behavior was recorded. On the fourth day, the mares were fitted with jugular cannulas, and blood samples were drawn for measurements. After the blood was collected, the mares were allowed 15 minutes of exercise in a 23 m x 23 m pen. The distance traveled by the mares was sketched on a trail map, and was later measured using a map wheel.

The results indicated no significant change in behaviors recorded at 48 hours between the housing conditions. Behaviors recorded included number of feeding bouts, total time spent feeding, and average grazing time. In open-field test trials the more confined mares demonstrated the most energetic activities (traveled farther, and trotted longer). The more confined mares also had a greater number of standing bouts, but spent less total time standing. The isolated mares traveled farther due to an increased motivation for movement resulting from isolated confinement (Mal et al 1991). The study concluded that the mare's behaviors were more affected by isolation, and reduced external stimuli than by confinement alone.

Within PMU housing, auditory, visual and tactile contact with stall mates is provided. As shown by the previous study, contact with like species contributes to the well-being of horses in confinement situations.

Mares on PMU farms are under extensive management at foaling. An experiment performed by Haas et al (1996) concluded that in an extensively managed mare herd; sire, mare age, body condition, and foal sex were not factors associated with foal mortality.

The ranch Haas et al (1996) conducted their study on placed pregnant mares in four large paddocks ranging form 10 to 30 ha. Paddock one contained 85, three year old mares, paddock two contained 77, four year old mares, and paddocks three and four contained 253 older mares split between each paddock. The results of the study indicate that foal death was significantly associated with dystocia, and failure of transfer of antibodies through the mother's colostrum, mostly due to poor mothering ability (Haas et al 1996). This particular farm had an excessively high (22%) foal mortality rate. These researchers stated that the high rate of foal mortality on this particular farm could have been avoided by a smaller number of mares per paddock, increasing the amount of observation times to assist mares experiencing dystocia, and to locate and isolate mares with poor mothering ability.

Certain groups are concerned about the small space the PMU mares must occupy during the urine collection periods, and the subsequent effects confinement may have on physical fitness. While no studies have been reported for equids, studies by Clark et al (1991) and Hetts et al (1992) investigated the impact of housing conditions on social isolation, exercise fitness, and behavior in beagle dogs. In the study by Clark et al (1991), 18 purpose-bred 9.5-10 month old female beagles were randomly placed into six groups of different housing conditions. Group A was similar to the situation of a family pet, in which the beagles were placed in an outdoor 6.1 x 9.1 m pen. Group B consisted of an outdoor run 1.8 x 6.1 m with a dog house; group C housing was an environmentally controlled 1.2 x 3.66 m run; group D housing was a 0.9 x 1.2 x .84 m laboratory cage; group E housing was a 0.9 x 1.2 x .84 m cage with treadmill exercise five times per week for 30 minutes; and group F was a smaller cage than recommended by the Animal Welfare Act (AWA). Conditions D, E and F were all in an environmentally controlled room. During the last week of the three month rotational period, conditions A, B, C, D, and F were exercised on a treadmill for 10 min / day at 75% of maximal heart rate for unconditioned dogs.

Results indicated that type of confinement had little effect on the activity of the mitochondrial enzyme succinate dehydrogenase. However succinate dehydrogenase activity of the gluteus medius muscle in group C was significantly higher than housing groups D, E, and F. Differences in heart rates only showed up between condition F and conditions A and B. Heart rates were higher in condition F after both the fifth and tenth minute of treadmill exercise. There were no detectable differences of heart rates in the dogs in other housing conditions. Plasma cortisol concentrations were also measured with no detectable differences among housing conditions. The results from this study indicate that pen size does not have a significant effect on physical fitness in dogs, with the exception of condition F which was a sub-standard size cage.

The study by Hetts et al (1992) examined the influence of housing conditions on beagle behavior. This study maintained the same housing conditions, and utilized the same number of beagles as Clark et al (1991). All housing conditions, except Group C allowed visual, and auditory contact between dogs. Group C dogs were isolated from other dogs and had limited contact with humans.

The results demonstrated that well-being should not be based entirely upon activity levels. Beagles that were socially isolated in Group C spent more time moving than beagles housed in other conditions. Group C beagles exhibited abnormal behaviors that were not displayed by dogs maintained in the other housing conditions. The beagles maintained in the larger pens were not necessarily more active all of the time, but were most active when they were socially isolated, or when a human attendant appeared. Beagles that were kept in conditions D and E spent more time grooming than dogs in conditions A and B. Treadmill exercise as used in group E did not alter behaviors compared to dogs in the same housing condition (condition D), with no treadmill exercise. It is clear from the conclusions of these studies that a dog's well-being can not be based solely upon cage size, or intensity of physical activity, but is also influenced by social isolation.

Dogs, like horses, are social creatures and are content when like species are within close proximity. Results of the beagle research can be compared to

PMU farms, as the mares are maintained in tie stalls that range from 1.07 to 1.52 meters from center to center depending upon the size of the mare. Even though these stalls are small, they allow auditory, visual, and tactile contact between species. Therefore, social isolation is not an issue for PMU farms.

Equine Behavior

According to non published sources, Animal Rights Activists are concerned about the foreign environment, and its confounding effects on PMU mares. The Recommended Code of Practice designed for the care of PMU mares, encourages that mares be tied in such a way as to permit lying comfortably, while also providing adequate room for stretching. According to Fraser (1992) systematic stretching is a function of a horse's well-being. It denotes bodily care and involves movements such as; flexion at the throat, arching of the neck, straightening of the back, movement of the tail, and full extension of hind or fore limbs. Fraser (1992) also noted that in any type of illness, stretching is a completely absent behavior.

Behavioral effects such as drinking, standing, eating, urinating and defecating, self-grooming, and walking were studied (Caanitz et al 1990) in 12 standardbred mares. The purpose of that study was to determine whether horses behaved differently on days they were exercised as opposed to days they were not exercised. Exercise was performed on a high-speed treadmill for 30 minutes. The speed at which exercise was performed ranged from 1.8 m/s to 9 m/s.

This study consisted of two elements. One element was between horses and the second element was within horses. The between horse element consisted of an exercised and a non exercised group which resulted in longer drinking bouts, decreased time spent urinating, and increased time spent lying down at night for the exercised group. There was no significant difference in overall time spent eating, walking or standing between the exercised and the non exercised group. The within horse element consisted of monitoring behavioral changes in the same horse on days that it was exercised as opposed to days no exercise occurred. The within horse group spent significantly more time drinking and urinating on exercise days than non exercise days. There were no significant differences in eating, defecating, self-grooming, and walking between exercise versus non exercise days. There was also no significant differences in duration of mouthing objects, vocalization, and social interactions between the two groups.

The results of that study indicated that all changes in behavior occurred immediately after exercise. If the horses were not exercised, their behavior remained unchanged. The major behavioral differences between stalled, penned, and free-ranging horses was time spent feeding. Horses with free access to forage spent most of their time grazing while confined horses spent the majority of their time standing. The conclusions of Caanitz et al (1990) suggested that forced exercise creates behavioral changes that would not otherwise occur if the horses were not forced to exercise.

Protein Requirements

Total Protein intake on PMU farms is increased from 8% to 10-11% crude protein during the last trimester of gestation (Horse Ration Balancer by Alberta Agriculture). Low levels of protein are fed to decrease the volume of urine produced and to decrease the quantity of water ingested. According to Lewis

(1995), pregnant mares need to receive at least eight percent crude protein the first nine months of pregnancy, and then increased to 11% crude protein in their diet by the 11th month of pregnancy just as is recommended by the Code of Practice for the Care and Handling of Horses on PMU Farms (1996). For a 600 Kg horse the NRC (1989) recommends an increase from 940 g of protein the ninth month of pregnancy to 1024 g of protein the 11th month of pregnancy.

Custalow (1991) researched protein requirements during exercise in the horse. Horses that are exercised regularly have increased nutritional requirements. Protein requirements are established for growing horses but little is known about the protein requirements of performance horses. The 1989 NRC protein requirements for a horse that receives light exercise (pleasure riding) is 9.8% on a dry matter basis.

Excess protein content of a diet increases water consumption due to increased nitrogen excretion, and is not recommended in situations where water is restricted, as occurs on PMU farms (Code of Practice 1996). Custalow (1991) noted that nitrogen retention is increased in horses during periods of exercise. There is also a noted increase in RNA concentration within skeletal muscle that may indicate increased capability for protein synthesis. This increase in protein synthesis and subsequent nitrogen retention may support skeletal muscle growth. In times of temporary nitrogen depletion, plasma amino acid concentration is maintained regardless of protein intake. A low protein diet of 5.5% crude protein for this reason is still adequate to maintain plasma amino acid concentrations. However, muscle hypertrophy in performance horses may indicate a need for increased protein concentration within a diet (Custalow 1991).

Miller-Graber et al (1991) examined six mature Quarter Horse mares in a cross-over design assigning each to either a control or a high protein group.

The control group received a 9% crude protein (CP) diet produced by diluting the 18% CP diet with cornstarch and wheat straw.

The low and the high CP groups were exercised on a motorized treadmill with an 11% grade. The exercise test consisted of a two minute warm-up at 1.4 m/s, 15 minutes at 4.5 m/s, and a 60 minute recovery period. Heart rates were monitored at rest, and at 5, 10, 15, and at the 5th minute of recovery by a direct-wire electrocardiogram. Blood samples were collected from three sites in four horses with exteriorized left carotid arteries, the right jugular vein, and the pulmonary artery at rest, 5, 10, and 15 minutes of exercise and at 5, 30, and 60 minutes of recovery. Venous blood was collected at the same time in the two horses without exteriorized arteries. Blood collection was facilitated by the use of indwelling catheters.

The results indicated that dietary protein level did not affect heart rate, oxygen uptake, or pyruvate. However, pyruvate and lactate levels were altered in the high protein group during the 5th minute of recovery. Pyruvate levels were higher, but lactate levels were lower than the control group, but overall no significant differences were detectable between groups. Blood lactate levels of 4 mmol/L is considered to be the anaerobic threshold (Miller-Graber et al 1991). When pyruvate production is increased above the capacity of the TCA cycle to metabolize it, then a net conversion of pyruvate to lactate and alanine is the result. The detectable differences in blood pyruvate and lactate in the 5th minute of recovery between groups may be explained by a decreased pyruvate dehydrogenase activity and/or NADH to NAD ratio. Blood lactate levels were not affected by the treatments, but the increased L:P ratio in the low CP diet group suggests that protein level may affect metabolism of pyruvate or lactate during exercise and the recovery period (Miller-Graber et al 1991).

High protein diets are associated with reduction in blood lactate levels in unconditioned horses (Custalow 1991). Blood lactate levels are measures used extensively in studies as an estimate of fatigue, and fitness level. It was concluded that increased protein intake not only facilitates muscle hypertrophy, but also decreases blood lactate concentrations during exercise. Fitness level can be assessed in PMU mares as compared with control or pasture mares by comparing blood lactate levels before, during and after exercise.

Physical activity and Blood Lactates

According to Lewis (1995) one of the best indicators of a horse's physical fitness is the increase in plasma lactate concentration, or the speed or exertion that increases the plasma lactate concentrations during exercise. Fitness does not have an effect on resting plasma lactate levels. For example, a horse that is conditioned may have the same resting plasma lactate levels as a horse that is obese and unconditioned. However, once a horse is exercised, plasma lactate concentrations will increase hyperbolically as a horse's speed or level of exertion increases.

Blood lactate levels can also be used to determine the threshold of anaerobic metabolism by a steady state of lactate production and oxidation. Valette et al (1993) examined the kinetics of blood lactate during constant exercise tests on a treadmill to determine VLa3 or VLa4 as markers of the anaerobic threshold. VLa3 and VLa4 are step tests required to elicit blood lactate concentrations of 3 mmol/L or 4 mmol/L at a certain velocity.

This study used four Anglo-arabian and one French saddle horse that were involved in three-day-event training. These horses were fed a diet that would fulfill their nutrient requirements for both maintenance and work.

Exercise tests were performed on a high speed treadmill with a 3.5% slope. Horses were given a warm-up period and then were galloped for eight minutes at a constant velocity of VLa3 and then 48 hours later at VLa4. After the exercise test the horses were given a 10 minute recovery period at 4.2 m/s. A catheter was used to take blood lactates every minute during exercise and during the recovery period.

The results of this exercise test at VLa3 were below 3 mmol/L. At times four to eight minutes the mean blood lactate led to the estimation of 2.25±0.40 mmol/L, or the anaerobic threshold. The VLa4 exercise test showed that at times seven to eight minutes blood lactates were consistent with the 4 mmol/L value, but blood lactate continued to rise from the 4th to the 8th minute with no steady state acquired. The VLa4 test is a test used to improve aerobic capacity because at this velocity the production of blood lactate passes beyond oxidation. For this reason VLa4 can be used to measure fitness in horses (Valette et al 1993).

Trained human athletes accumulate blood lactate at a slower rate during exercise and remove lactate more quickly than sedentary subjects (Rainger et al 1994). Rainger et al (1994) utilized 14 thoroughbred horses to measure blood lactate disappearance in a 40 minute recovery period, to assess the relationship between blood lactate accumulation during exercise and lactate disappearance during recovery, and lastly to reveal the relationship between these measures and the state of training or fitness of the horse. These 14 horses were divided into a trained and a detrained group. The trained group had previously ran a race within 10 days of this study and were considered fit for racing. The detrained group had spent the previous four months in a paddock where walking was permitted along with occasional cantering bouts.

All 14 horses underwent a step wise exercise test on a treadmill set at a six degree slope. Prior to exercise, each horse received a 14 gauge catheter inserted into the jugular vein where 2.5 ml blood samples were collected during the last 15 minutes of exercise and at 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 and 40 minutes after exercise. Oxygen uptake was also analyzed during the last 15 seconds of exercise at each speed, by a loose-fitting gas collection mask.

The results of this study indicate that the trained horses, like the trained human athletes, had lower lactate concentrations during exercise regardless of exercise intensity. Blood lactate concentration was also greater throughout the 40 minute recovery period in the detrained group. There were only moderate correlations during and after exercise between lactate variables. Post exercise disappearance of lactate varied between horses, thus the disappearance of lactate during the 40 minute recovery period was independent of fitness level and varied widely between individuals. In some horses the function was linear but in others it was exponential. The results in this study may have been influenced by the handling of the horses after exercise, which is shown to effect the disappearance of lactate (Rainger et al 1994).

The high speed treadmill has tremendously advanced the study of equine exercise physiology. Standard exercise tests (SET) are commonly used to study training effects on horses. Standard exercise tests are exercise bouts of equal intensity (Harkins et al 1991). Harkins et al (1991) developed a study to demonstrate the effects of interval training (IT) using a high speed treadmill and SETs to determine whether IT improves cardiovascular fitness in thoroughbred race horses. Nineteen thoroughbred horses were used, all of which had racing or training experience, and had not been in training for at least six months prior to this study. All horses underwent a 20 week IT training period. Heart rates were monitored using an on-board heart rate monitor. Two electrodes were placed on the girth strap and were attached to a shaved area using generous amounts of electrode gel on both the sternum and on the left side of the horse's chest, midway between the sternum and withers. The heart rate was transmitted via telemetry from a transmitter in the girth strap to two receivers mounted on the treadmill.

The results of this treadmill study indicated that Pre-training SET heart rates performed during week five varied between 142-187 beats/min between horses. The Post-training SET performed during week 20 demonstrated less than a 10 beat/min heart rate improvement in three of the horses, while 16 horses showed a 10-27 beat/min improvement in mean heart rate. The authors of that study did not believe that a 3-week introductory period, as used within the study, are necessary to adapt horses to the treadmill.

Rodiek et al (1987) examined the effect of two treadmill conditioning programs on nine mature quarter horse mares. The first conditioning program consisted of interval-type work with intermittent rest programs and the second conditioning program consisted of a continuous work regime. The continuous exercise group trotted on a treadmill set at a 3.0% incline, while the intermittent group trotted at the same speed of 3.3 m/s on a treadmill set at a 9.0% incline. Treadmill incline was proportional to trotting time in order to maintain equal physical work between groups as shown by the equation $d = t * v$ (d=distance, t=time spent trotting, v=vertical grade). The study was divided into two five-week segments with a SET administered at the beginning and at the end of each part.

The results indicated that the intermittent group had consistently higher heart rates than the continuous group, but overall heart rate decreased between the first and the last SET in both groups. There was no significant difference between conditioning groups and cardiac output or stroke volume. Lactates were not significantly different between the two exercise groups, however, lactate concentrations decreased significantly between SETs 1 and 2 for both groups indicating improved oxygen extraction or utilization. The physiological mechanisms by which the lactate accumulation decreased between the two SETs is not thoroughly understood. Studies in human endurance athletes has shown an increase in skeletal muscle capacity for aerobic metabolism. This is indicated by increased muscle cell mitochondrial size and number, along with increased levels of mitochondrial enzymes. This mitochondrial adaptation to endurance activities has also been demonstrated in horses. This study resulted in no physiological adaptations to submaximal exercise in either the continuous or intermittent conditioning programs. The increased heart rate values in the intermittent group as compared to the continuous group were thought to be due to individual horse variation, as the differences were most noticeable within the first SET and least noticeable in the last SET.

Rodiek's et al (1987) study lasted two and a half months. This was an adequate period of time to elicit changes in blood lactate and heart rate values. Pregnant mares in this study are not going to be exercised, however, a three month research period should be adequate in determining whether a change in physiological parameters such as heart rates and blood lactates develop.

Chapter III

Materials and Methods

Ten pregnant mares from the Oklahoma State University horse farm were utilized for this experiment. The control group, consisting of five mares, was maintained on a 15 acre pasture with ad libitum access to hay and water. The other five mares, or the experimental group, were confined within a simulated PMU barn. The simulated PMU barn consisted of tie stalls with the same dimensions listed in the Recommended Code of Practice for the care and Handling of horses on PMU Farms. For example, a mare weighing 590.7-771.8 kilograms (kg) is confined in a 1.37 m tie stall measured from center to center. The simulated PMU barn had an open-faced south side, and therefore was not temperature regulated. In January, the south side of the barn was covered with a tarp to help prevent freezing of water, and urine collection tubes.

The confined mare's daily urine production was collected and measured in liters twice daily. Water consumption along with hay and grain refusals were recorded in a daily log (table I). These mares were watered two to three times a day, or as needed, and were given access to 2.72 kg, and later increased to 3.18 kg of prairie hay, measured by a scale each feeding. All ten mares received 1.81 kg of a pelleted ration (table II) fed at 7:00 am and 5:00 pm. The ration was increased to 2.27 kg per feeding the last month of the study to compensate for 70 percent of fetal growth (Code of Practice 1996), along with an increased digestible energy requirement of 23.3 Mcal occurring during the last trimester of gestation (600 kg horse NRC 1989). Mineral balance was calculated using average feed intake throughout the study resulting in 4.09 kg of concentrate intake/day and 6.36 kg/day of hay.

Table I

Daily PMU Data

Horse	Month	Hay Refused* (lb.)	H2O Intake (gal)	Urine Output (L)
1	Oct	0.793	2.479	2.252
1	Nov	0.671	2.521	1.950
1	Dec	0.351	1.556	1.637
1	Jan	0.802	2.125	1.589
2	Oct	0.369	2.403	2.221
2	Nov	1.350	2.763	2.093
2	Dec	0.244	2.609	1.644
2	Jan	0.107	2.205	1.463
3	Oct	0.965	3.403	2.622
3	Nov	0.703	3.692	2.254
3	Dec	0.334	3.778	1.660
3	Jan	0.675	2.929	1.636
4	Oct	0.876	2.597	3.350
4	Nov	0.410	2.522	2.541
4	Dec	0.050	2.903	2.694
4	Jan	0.361	2.348	2.700
5	Oct	0.406	2.160	1.674
5	Nov	0.652	2.425	1.620
5	Dec	0.373	2.694	1.274
5	Jan	1.329	2.339	1.300

Table I. * Pelleted concentrate was not refused by the confined mares throughout the three month collection period.

TABLE II

PMU DIET COMPOSITION

Ingredient	% Concentrate	% of Diet
Concentrate		36
Shelled Corn, Ground	67.50	
Soybean Meal	14.95	
NRC Cottonseed Hulls	15.75	
Trace Mineralized Salt	0.44	
Limestone	0.70	
Dicalcium Phosphate	0.66	
Prairie Hay		64

Treadmill

Prior to and immediately following the confinement period, both groups of mares were trotted on a high speed treadmill at a rate sufficient to elicit a heart rate of 160 beats per minute. This is the heart rate considered by most exercise physiologists to be the anaerobic threshold (Lewis 1995). Heart rates were recorded by an on-board Polar Electro System that allowed continuous data collection during exercise and during the first five minutes of the recovery period. Two self-adhesive electrodes were attached to shaved areas slightly below the point of the wither on the left side and on the sternum. Information from these electrodes was transmitted via telemetry to a receiver attached to the treadmill (Harkins et al 1991). The mares were given a warm up period of one minute and then gradually increased to a speed of 12 to 15 miles per hour, depending upon the mares physical capability. The time the mares spent on the treadmill ranged from 8.4 to 12 minutes contingent upon the time required to elicit a heart rate of 160 beats/minute. Blood lactates were taken from the jugular vein prior to exercise, immediately after, and then at 5, 10, 20, 40, and 60 minutes after treadmill exercise using vacutainer syringes with 20 x .5 inch gauge needles. Heart rates were recorded simultaneously using a stethoscope during the last 55 minutes of the recovery period. Mares were allowed to remain on the treadmill for a five minute recovery period, at which time the post and 5 minute heart rates and lactates were collected. The mares were then tied to a trailer to reduce variation in heart rate and lactate values that may occur if the mares were cooled by walking upon the completion of the exercise test (Rainger et al 1994). Blood lactates were refrigerated after collection and then analyzed on a COBA MIRA/MIRA S[1] analyzer utilizing a Sigma Diagnostics Lactate Procedure[2] .

Mineral Balance

Mineral balance was assessed via collection of fresh fecal samples. Samples were collected from the confined mares maintained on a 0.20% chromic oxide concentrate prior to and at the end of the study. Chromic oxide is an indigestible marker used to determine fecal output (Holt 1992). The confined mares were fed the chromic oxide diet for seven days prior to the collection period, and were maintained on the diet throughout the three day collection period. Results utilizing a three day collection period, as compared to a seven day collection period, using sixteen lactating cows indicated no significant differences between the length of the collection period and corresponding chromic oxide results (Hattan et al 1970).

Urine samples were collected twice daily via urine collection boots attached by a suspension hitch system, the same system utilized on PMU farms. These harnesses were initially attached using surgical tubing, and were later replaced with bungie rope. The urine collection boots were hung just below the mare's vulva. When the mare would squat to urinate, the collection boot would be in the appropriate position to collect urine. The urine would then flow from the boot into a rubber tube, 3/4 inches in diameter, and into collection buckets placed in front of each stall. A percentage of the total urine volume was collected and acidified with 1.5 ml hydrochloric acid (HCL) to minimize bacterial

1 COBAS MIRA, COBAS MIRAS, Roche Diagnostic Systems Inc., Branchburg NJ.
2 Sigma Chemical Company d.b.a., Sigma Diagnostics, St. Louis Mo.

growth. The normal urine pH of grazing horses is 7.9, but when supplemented with grain the pH is slightly more acidic measuring 7.4 (Wood et al. 1990). Urine samples were frozen immediately after collection, composited into 50 ml portions and analyzed for Ca, P and Mg on a EKTACHEM 700 analyzer[3] .

Fresh fecal samples were collected at different times throughout the three day collection period to exclude diurnal variation that is known to occur in sheep, goats, cows, and chickens (Saha et. al. 1991). The fecal samples were collected at the following times throughout the three day collection period:

Day 1	Day 2	Day 3
9:00am	7:00am	11:00am
1:00pm	1:00pm	3:00pm
9:00pm	11:00pm	5:00pm
1:00am	3:00am	7:00pm

Immediately following collection each fecal sample was frozen. Fecal samples were allowed to thaw overnight and then placed in a 55 ' F oven for 72 hours. Once the samples were dryed, they were ground in a Regal grinder. Five gram samples were taken from each of the 12 fecal samples to form a composite sample. The composite samples were then analyzed for calcium, phosphorus, and magnesium by the DHI Forage Testing Laboratory's ICP[4] .

Chromic oxide values were assayed as described by the method of Michael Galyean (1990) using an atomic absorption spectrophotometer[5] .

Behavioral observations

3 KODAK EKTACHEM, Model 700, Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY.

4 ICP Gerald Ash, Fisher Scientific Atom, Model 1000.

5 Perkin-Elmer Atomic Absorption Spectrophotometer, Model 4000.

Behavior was not a statistically measured test criteria for this study, however, some aspects of behavior were observed. Feeding behavior was monitored and recorded as indicated by the amount of grain and hay refused (Table 1). Stretching and body language were also monitored. The confined mares were groomed once a week to promote well-being.

Statistical Analysis

Statistical analysis was performed on treadmill results and analyzed by Analysis of Variance for Repeated Measures (ANOVA). Between and within group effects were analyzed for the two treadmill periods. To reduce statistical variation, mares were trotted on the treadmill during the second treadmill period for the same length of time as the first treadmill period. Statistics were computed on a Statistical Analysis System (SAS) for Windows version 6.11. Mineral balances were also computed on SAS, only a Student's T test was utilized to test the differences of means between the first and second fecal and urine collection. All data were analyzed with a significance declared at the $p<.05$ level.

Chapter IV

Results

Mineral Balance Results

The analysis of the feed composition tested by the DHI Forage Testing Laboratory is shown in table III. The confined mare's mineral balance increased from the first month of collection to the third month of collection as shown by figure I. Calcium balance increased significantly ($p<.05$), as did magnesium. Phosphorus balance increased numerically from the first to the third month, however the increase was not significant.

Treadmill Results

Analysis of Variance procedure for testing the hypothesis of between subject effects indicated that during the first treadmill period, a significant ($p<.05$) difference occurred between horses, and their corresponding heart rate and lactate values, regardless of groupings. There was also a significant effect between treadmill periods and heart rate and lactate values. Pre exercise, post exercise, 5, 10, 20, 40, & 60 minute time periods coincided with significantly different blood lactate and heart rate levels. However, there was no significant difference when comparing interactions between groups and times the lactates were taken, or between groups and the two treadmill periods, or between group by period by time interactions (Table IV).

Table III

CHEMICAL COMPOSITION OF PMU DIET

Feed	As Sampled %	Dry Matter %
DM	85.2	
Crude protein		16.60
Crude fat		3.10
Calcium		0.72
Phosphorus		0.52
Magnesium		0.17
Crude fiber		6.60
Hay	**As Sampled %**	**Dry Matter %**
DM	92.9	
Crude protein		5.40
Crude fat		2.10
Calcium		0.37
Phosphorus		0.06
Magnesium		0.17
Crude fiber		34.2

Table III. DHI Forage Testing Laboratory results

Figure 1
MINERAL BALANCE OF MARES CONFINED FOR
THREE MONTHS

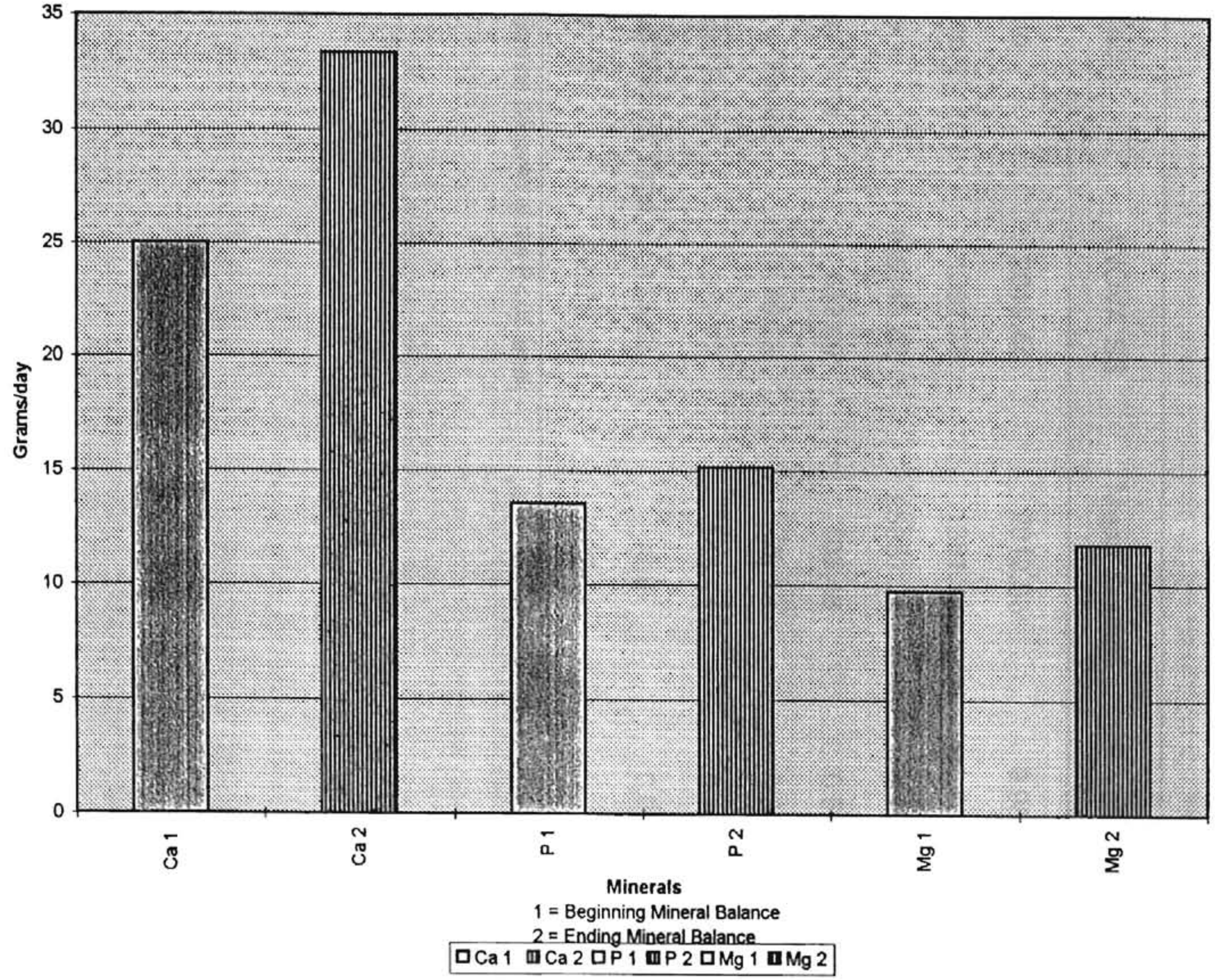


Table IV

Results of Analysis of Variance for Between Subject Effects of Heart Rates and Lactates

Subject	ANOVA SS	Mean Square	F Value	Pr>F
Group	2019.66	2019.66	9.98	0.0020
Period	1381.73	1381.73	6.83	0.0102
Time	155143.42	25857.24	127.75	0.0001
Group*Time	632.89	105.48	0.52	0.7913
Group*Period	76.13	76.13	0.38	0.5409
Period*Time	872.72	145.45	0.72	0.6354
Group*Period*Time	113.82	18.97	0.09	0.9969
Error	22670.00	202.41		

Significance declared at the P<.05 level.

The ANOVA test for within subject results (Table V) indicated a significant difference in times the lactates were collected, however no significant effects of within subjects were indicated when time by group, time by period, or time by group by period interactions were compared. The results of the treadmill exercise trial are demonstrated in figures two through nine.

Analysis of Variance procedure for Tukey's Studentized Range (HSD) indicates a significant difference ($P<.05$) in heart rates, but no significant difference was obtained in lactate values between control and confined groups (Table VI). This analysis indicated no significant difference in lactate levels between the first and second treadmill periods. However, there was a significant difference found in heart rate levels between the first and second treadmill period, as well as significant differences in times the samples were collected.

Behavior

Feeding behavior was not affected by confinement during this study. None of the experimental mares refused their grain throughout the three month study. The majority of the hay provided was also consumed (table I). Prior to feeding, the mares showed excitement by pawing, and vocalization. Their body language consisted of ears back, and head nodding toward their stall mates. An occasional kick, or leaning against the stall panels was also observed, however, no idiopathic behaviors were noted. The confined mares displayed the same type of behavior stalled, or penned horses exhibit during feeding.

Stretching behavior is an indication of well-being, as it is completely absent during any kind of illness (Fraser 1992). The confined mares

Figure 2
PMU BEGINNING TREADMILL HEART RATE RESULTS

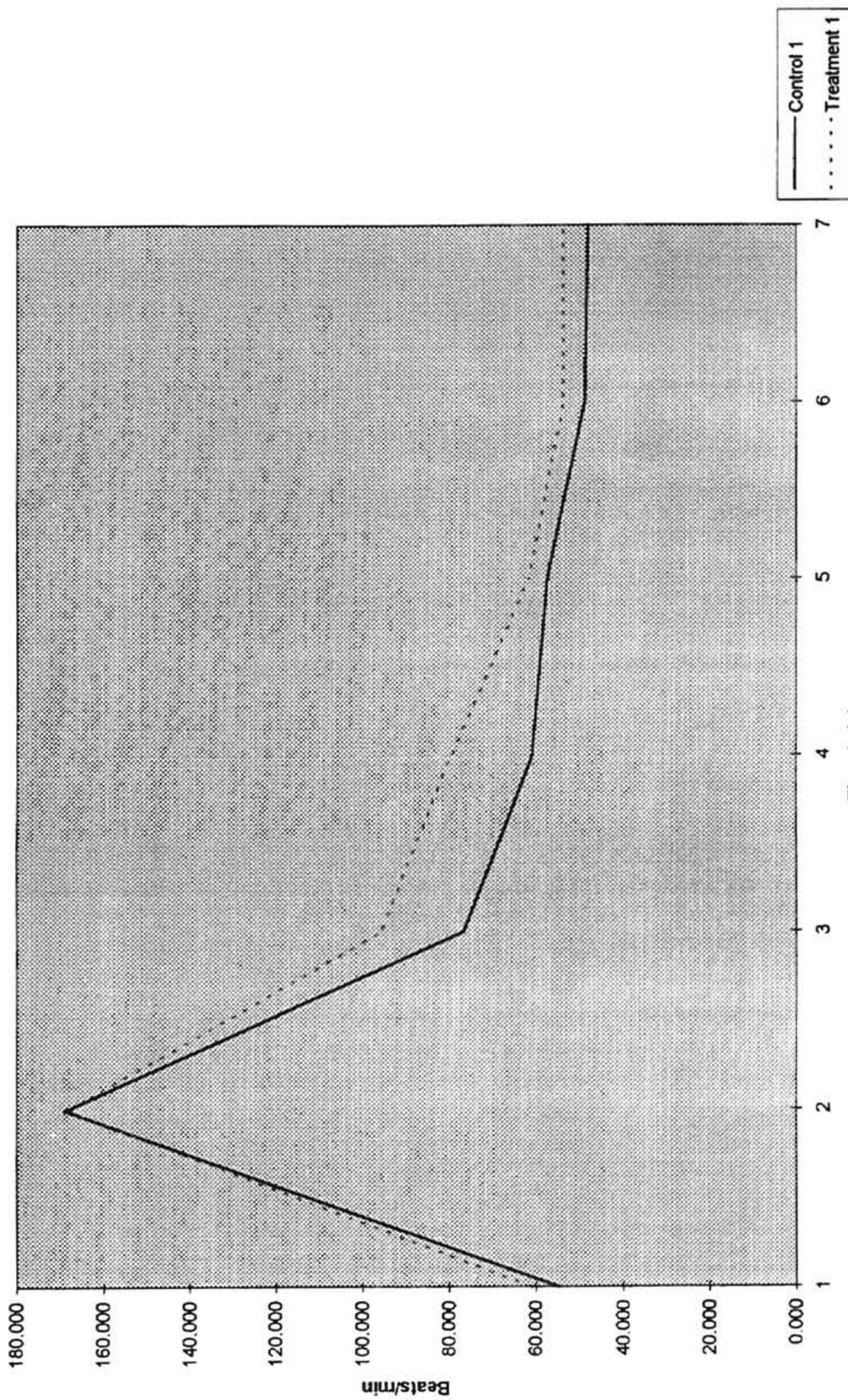
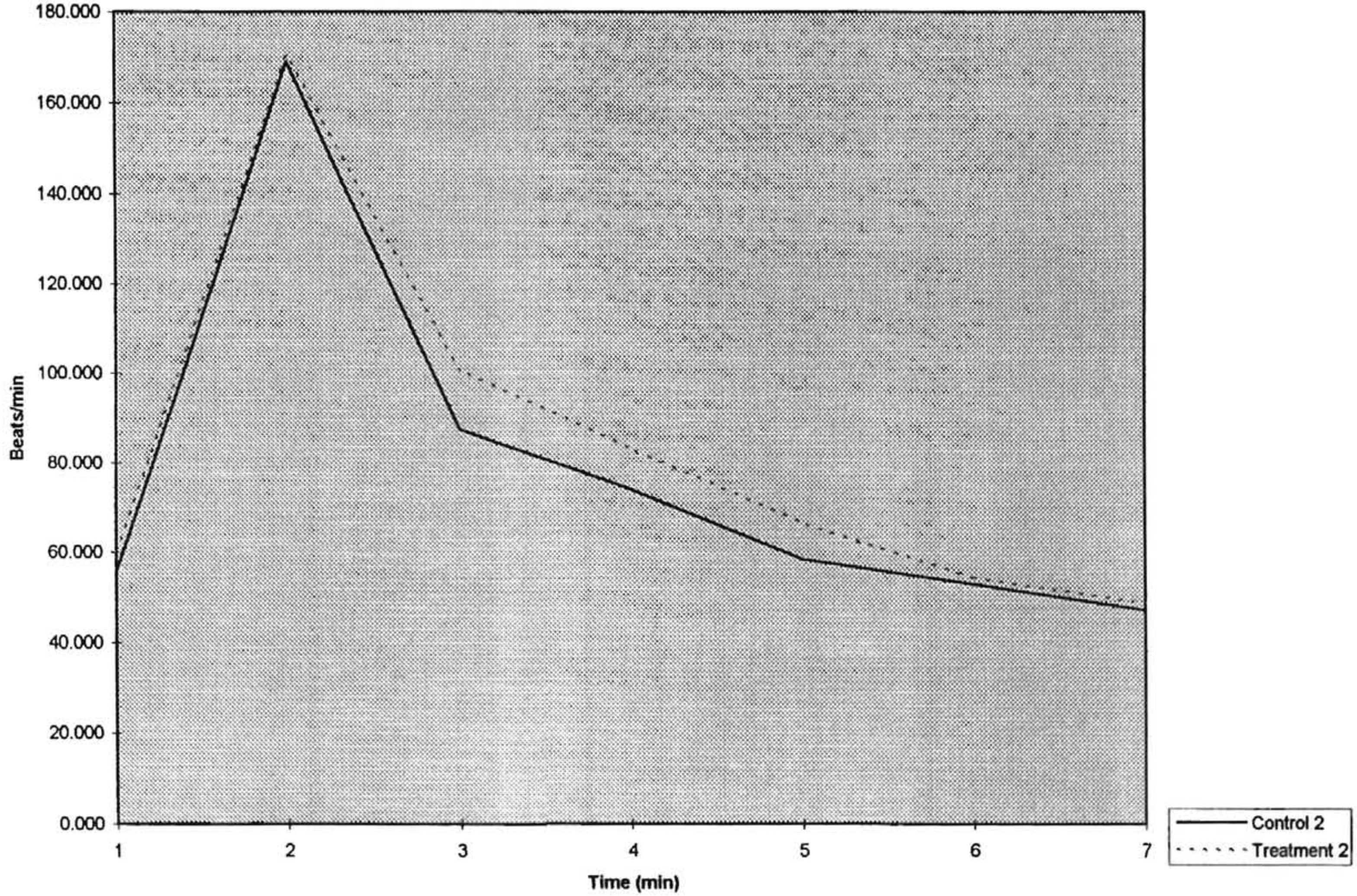


Figure 3
PMU ENDING TREADMILL HEART RATE RESULTS



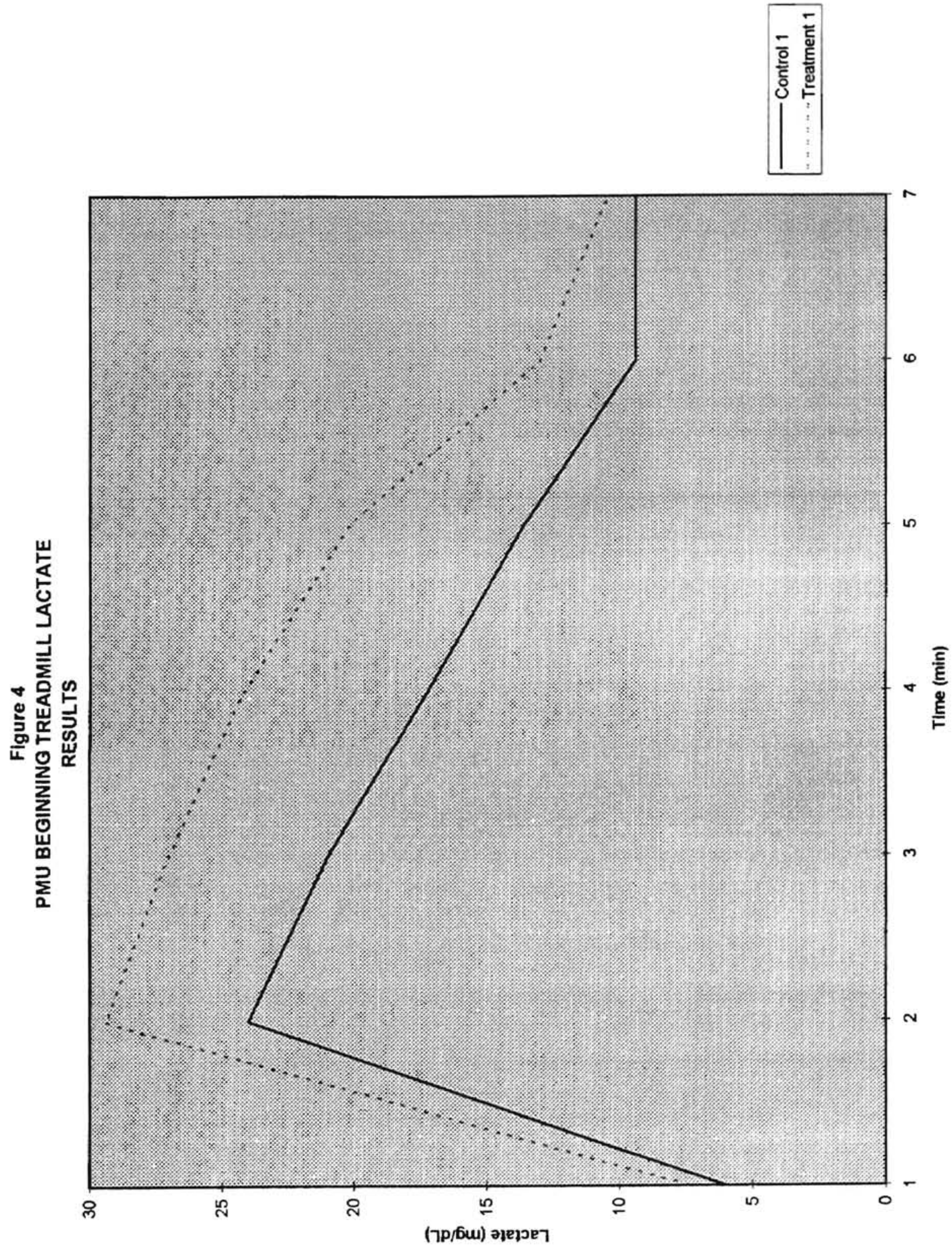


Figure 4
PMU BEGINNING TREADMILL LACTATE
RESULTS

Figure 5
PMU ENDING TREADMILL LACTATE
RESULTS

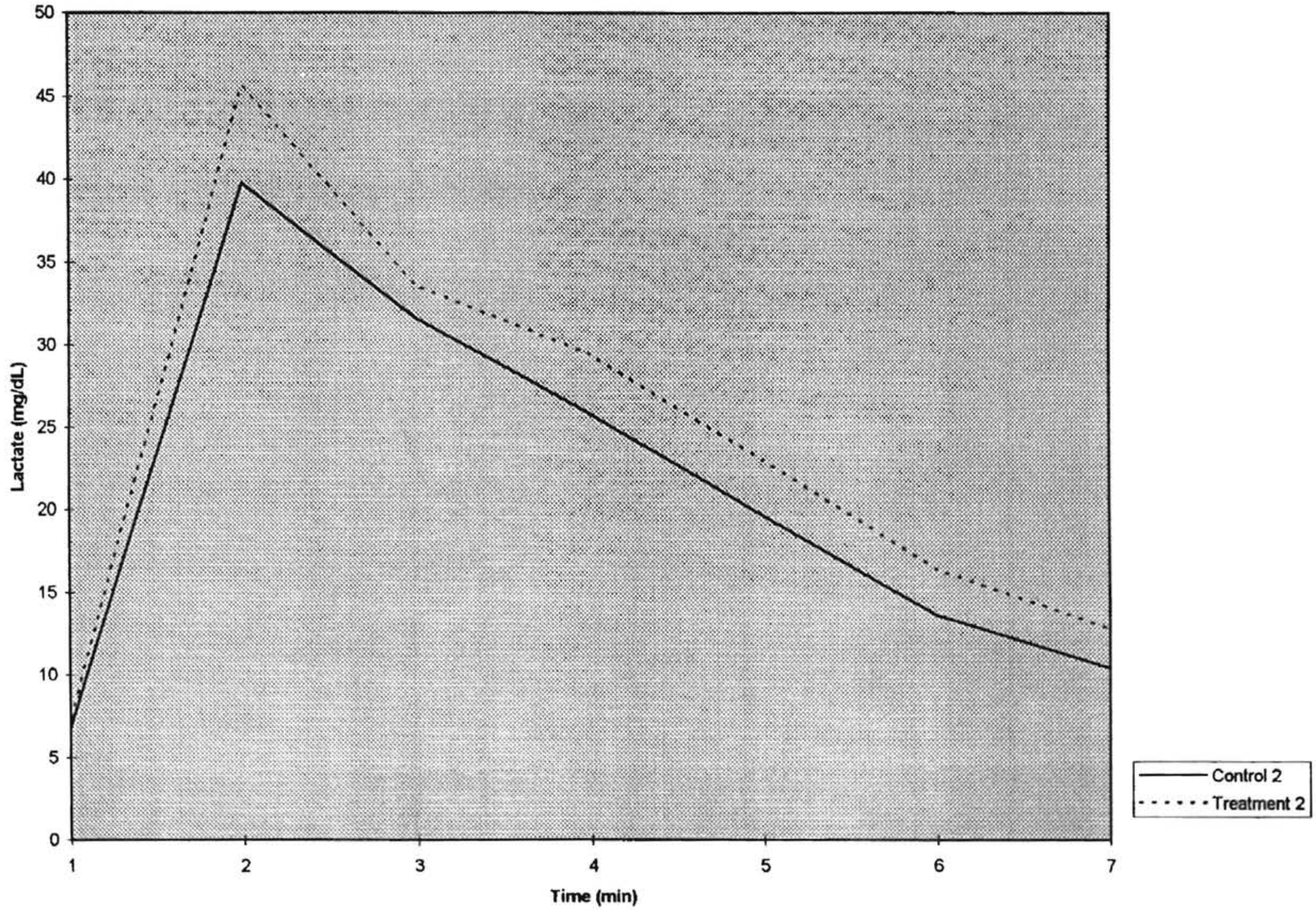


Figure 6
PMU TREADMILL HEART RATE CONTROL
RESULTS

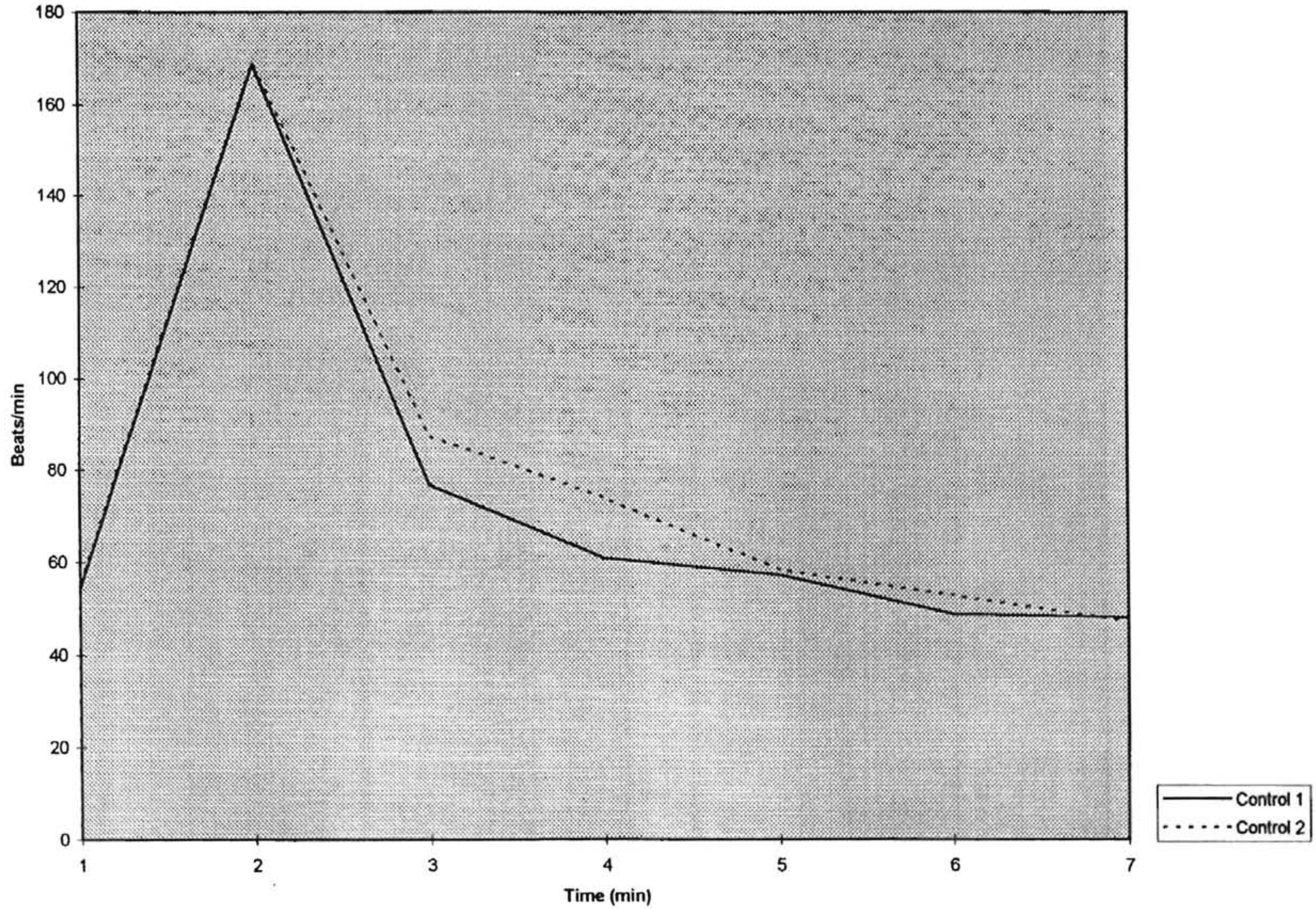


Figure 7
PMU TREADMILL HEART RATE TREATMENT RESULTS

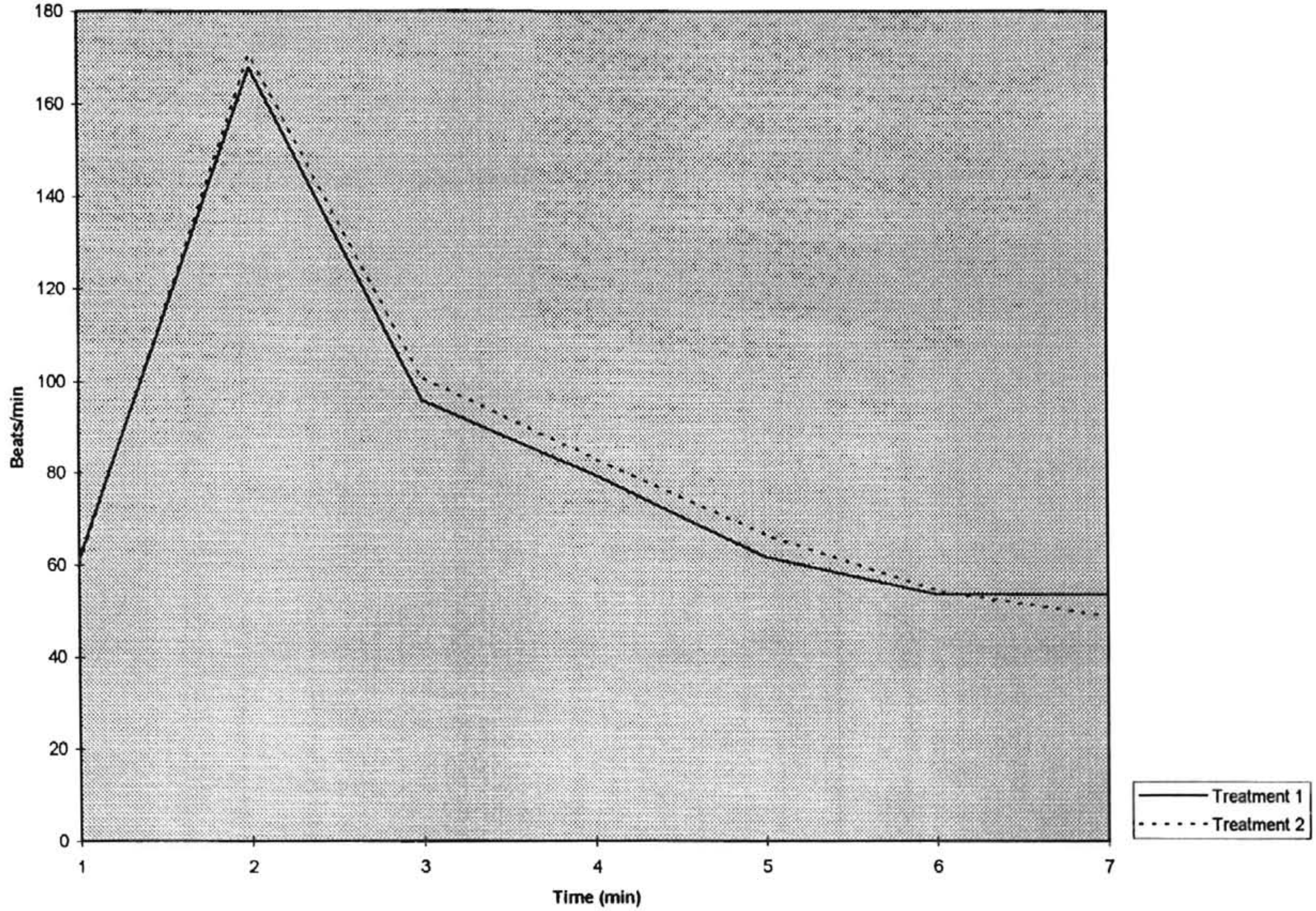


Figure 8
PMU TREADMILL LACTATE CONTROL
RESULTS

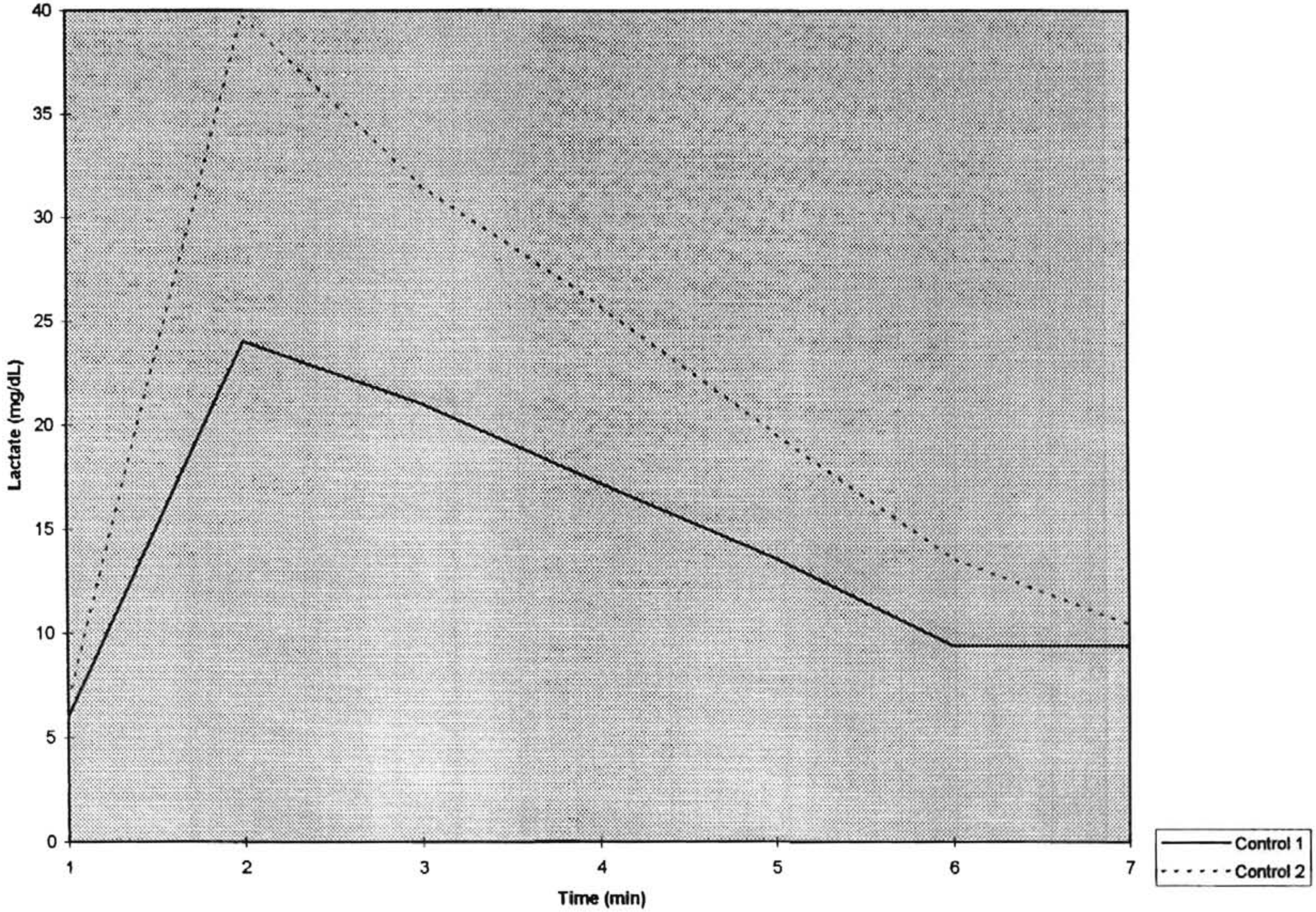


Figure 9
PMU TREADMILL LACTATE TREATMENT RESULTS

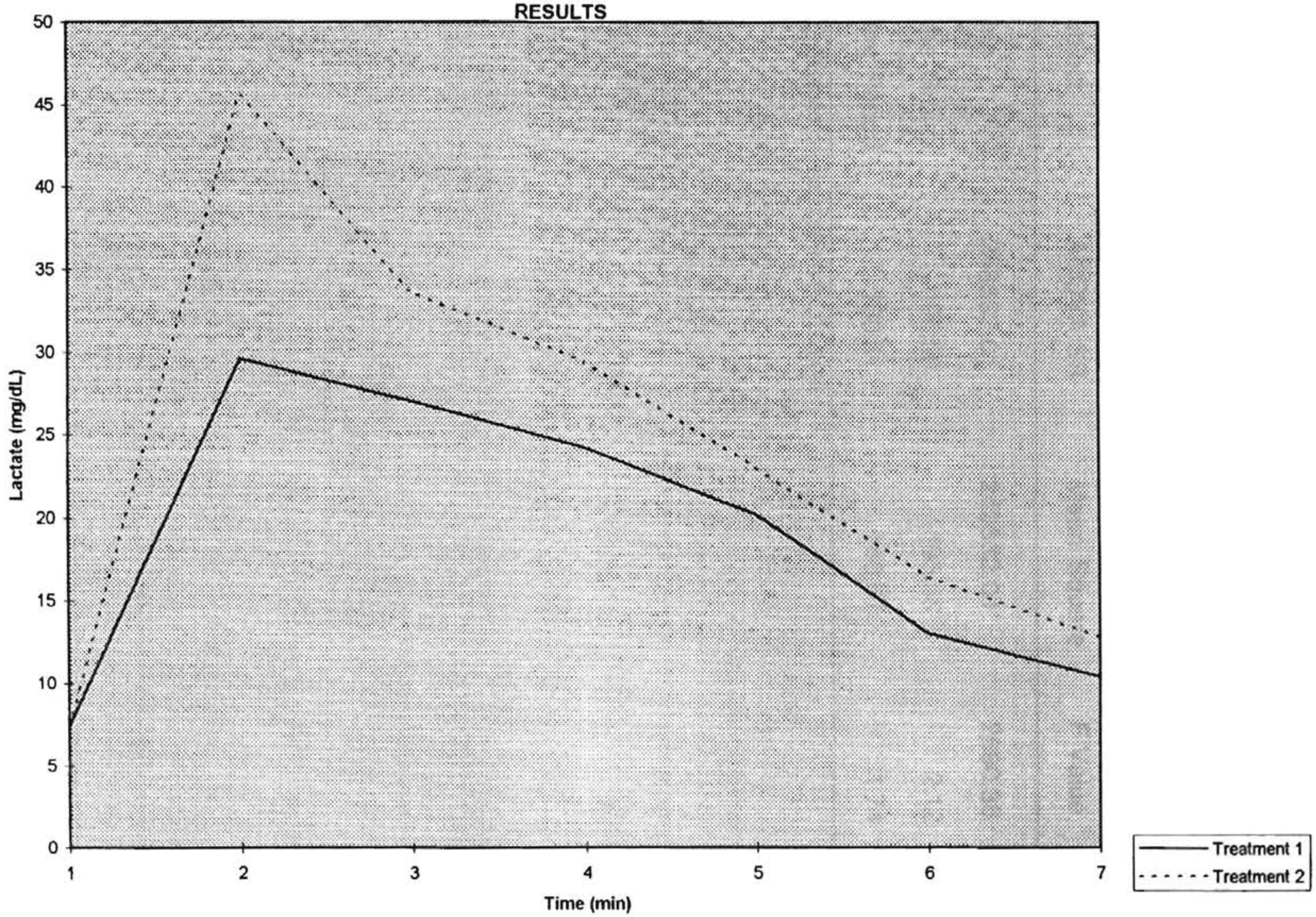


Table V

Results of Analysis of Variance Univariate Tests for Within Subject Effects of Heart Rates and Lactates

Subject	ANOVA SS	Mean Square	F Value	Pr>F
Time	248532.01	248532.01	2690.36	0.0001
Time*Group	195.56	195.56	2.12	0.1485
Time*Period	160.51	160.51	1.74	0.1901

Significance declared at the P<.05 level

Table VI

INFLUENCE OF GROUP, TREADMILL PERIOD, AND TIME ON LACTATE AND HEART RATE VALUES

Group	Heart Rate	Lactate
Treatment	82.69^{a}	21.43^{a}
Control	75.64^{b}	17.73^{a}
<u>Treadmill Period</u>		
First	77.70^{a}	16.60^{b}
Second	80.63^{a}	22.56^{a}
<u>Time (min)</u>		
Pre	57.85^{de}	6.90^{d}
Post	169.0^{a}	34.75^{a}
5	90.15^{b}	28.30^{ab}
10	74.40^{c}	24.15^{abc}
20	60.95^{d}	19.10^{bcd}
40	52.40^{de}	13.10^{cd}
60	49.40^{e}	10.75^{d}

Superscripts with different letters are means that differ significantly ($P<.05$).

displayed stretching behavior predominantly during the morning hours. Their stretching behaviors consisted of hind and foreleg extension, as well as yawning. The mares did not demonstrate abnormal behaviors during the three month confinement period which indicates their well-being was not affected (Mal et al 1991).

Chapter V

Discussion

Mineral Balance

Lewis (1995) recommends an increase of 10-20%, during the last trimester, in calcium and phosphorus requirements above that of the NRC's to reduce the risk of developmental orthopedic disease. Lewis (1995) suggests calcium levels of .50% DM and phosphorus levels of .30% DM. Calcium levels fed during this study were .53% DM and phosphorus levels were.25% DM. Although phosphorus levels are slightly lower than Lewis (1995) recommends, the calcium to phosphorus ratio is at the recommended 2:1. PMU farms have difficulty obtaining a ratio of 2:1 because of the quantity of grain fed. Grain is known to obtain higher levels of phosphorus than calcium, for this reason PMU farms have a calcium to phosphorus ratio of 1:1. The quantity of Calcium, phosphorus and magnesium in the ration was 52.9, 25.06, and 17.75 grams per day. Lewis (1995) recommends that a 600 kg (1320 lb) pregnant mare receive 41g of calcium, 30 g of phosphorus, and 10.3 g of magnesium at the ninth month of gestation. The diet fed in this study was adequate to maintain body condition, and consequently a positive mineral balance.

The positive mineral balance indicated by the bar graph shown in figure 1 indicates a significant increase in calcium and magnesium ($p<.05$), although the increase in phosphorus was not statistically significant, there was still a numerical increase in phosphorus balance ($p<.0555$). The significant increases in the mineral balances of these mares was expected primarily because 70% of fetal growth and development occur during the final three months of gestation.

Treadmill

During the first exercise test there was a significant difference between horses and their corresponding heart rate and lactate values. This was expected due to variation between individual horses. To eliminate variation in results between the first and the second treadmill periods, the mares were trotted for the same length of time, and at the same speed as occurred during the first treadmill period.

The three month confinement period had no significant influence on the experimental mares fitness level. Three months of confinement did not significantly increase heart rate response to treadmill exercise in confined mares, however, their lactates did increase from a peak of 39 mg/dl during the first treadmill period to 46 mg/dl during the second treadmill period. This rise in lactate would indicate that the confined mares lost fitness, however, the control group's lactates rose from a peak of 23 mg/dl during the first treadmill period to 40 mg/dl during the second treadmill period. There were no significant differences between the two treadmill periods when comparing the confined and the control group's heart rate and lactate values. The increase in lactates that occurred in both confined and control mares during the three month period is attributable to later stages of gestation, and increased body weight in most of the mares (table VII). Although most of the confined mares either lost or did not gain weight during the three month period, it did not affect foal weights or parturition length as shown by table VIII.

The implications of this study indicate that PMU horses do not lose fitness during urine collection periods. The confined mares were not turned out for exercise during the entire three month collection period, even so, there was no

Table VII

Weights of Control and Confined Mares

Confined	Starting Weight (kg)	Finishing Weight (kg)	Weight Gain (kg)
1	625.6	639.2	13.6
2	607.5	603.8	-3.7
3	603.8	603.8	00.0
4	637.4	650.1	12.7
5	662.8	653.8	-9.0
Control			
6	622.0	656.5	34.5
7	666.5	709.1	42.6
8	605.6	635.6	30.0
9	631.1	649.2	18.1
10	476.7	521.2	44.5

Table VIII

Parturition Data

Confinement Horses	Due Date	Actual Date	Gestation Length	Parturition (min.)	Foal Weight
1	3/25	3/23	335	8.0	123
2	4/01	4/04	340	20.0	115
3	2/18	2/13	332	-	101
4	-	3/07	-	-	103
5	3/31	4/01	340	25.0	121
Control Horses					
6	3/29	3/31	339	5.0	131
7	3/22	3/27	341	20.0	127
8	2/22	2/25	340	15.0	100
9	3/15	3/26	348	-	-
10	-	-	-	-	-

Table VIII. Where a dashed line occurs, parturition information was not available or the mare did not foal by the time this paper was completed.

significant differences in heart rates or lactates when compared to the control mares which were allowed unrestricted movement within the confines of a 15 acre pasture.

The majority of PMU collection periods are 5 to 6 months in duration, nearly twice as long as the present study, however 97.7% of the farms surveyed by NAERIC (1994) have established exercise programs. Of the 97.7% of the farms surveyed, 49% have individual exercise regimens to meet the specific needs of each mare, and 27% have specific exercise programs that are supplemented with individual exercise programs. The most widely utilized, established exercise programs that are also designed to meet the special needs of certain mares are; a rotational basis (11%), a weekly basis and as individual horses require (4%), a monthly basis and as individual horses require (4%), a rotational, monthly basis and as individual horses require (3%), and finally a 2% rotational, weekly basis and as individual horses require. Twenty one percent of the PMU farms have exercise programs that meet the needs of the herd as a whole. These programs consist of a rotational basis (6%), weekly basis (4%), rotational, weekly basis (3%), monthly basis (3%), and a rotational, monthly basis (2%). According to the results of NAERIC's survey and the current study's results, mares are not losing fitness. The exercise these mares are receiving on PMU farms, if any, is adequate to maintain the mare's physical fitness and well-being.

Well-being

Fitness is defined as suitability or well-being by Webster's New World Stedman's Concise Medical Dictionary (1987). The PMU mares are suitable for foaling. All mares on PMU farms are allowed to carry their foals to term, as were the mares in this study. The confined mares delivered their foals without experiencing dystocia, and are by definition "fit" (Table VIII).

Well-being when applied to animals generally includes good health and husbandry (Hetts et al 1991). The confined mares were all in good health at the beginning and at the end of the study. The confined mares received excellent care throughout the three month study, they were de-wormed and vaccinated at the beginning and again at the end of the study. Mares were watered as needed, fed and manure cleared twice daily, bedded in thick bedding, and groomed once a week. The animal husbandry the confined mares received was adequate to ensure well-being.

Many individuals feel that PMU mares are not experiencing a significant measure of well-being due to restricted movement. Hetts et al (1992) determined that activity level is not a good indication of well-being. Isolated Beagles when compared to non-isolated Beagles, exhibited the most strange behaviors and the highest level of activity. Mal et al (1991) demonstrated that social isolation in mares also resulted in the greatest motivation for movement as compared to mares who were confined, but not isolated. Mares on PMU farms are not socially isolated, as they are allowed visual, auditory, and a small degree of tactile contact.

Wyeth-Ayerst the producer of Premarin, has chosen certain equine welfare organizations to investigate allegations of neglect made by certain organizations concerning the welfare of PMU mares. These chosen organizations include; the American Association of Equine Practitioners, the Canadian Veterinary Medical Association, and the International League for the Protection of Horses. Each organization selected a representative to visit PMU farms in North Dakota, Alberta, Manitoba, and Saskatchewan during the 1996-97 collection season. This team of representatives reported its findings to the

Canadian Farm Animal Care Trust (CANFACT), which in turn reported that it is impressed by the improved welfare of horses on PMU farms, and believes that PMU producers, like most livestock farmers are genuinely concerned about the care and welfare of their animals. This group also reported that evidence supporting allegations of neglect occurring on PMU farms does not exist (Jones 1996).

Behavior

Changes in behavior patterns is one of the first indications that an animal's perception of its environment is changing. Changes in behavior are manifested in different motivational states, and can be used as an indicator of well-being (Mal et al 1991). In this study feeding, stretching and grooming behaviors were monitored. Feeding behavior was the only measured behavior within this study, and was unaffected despite the mares confinement and lack of physical activity.

Conclusions

The confined mares exhibited a definite measure of well-being throughout this study, however future studies examining and quantifying behaviors of PMU mares as well as measuring plasma cortisol levels may be warranted. In addition, a study researching the effects of long term confinement on soundness in mares would also be beneficial in assessing the well-being of mares on PMU farms. Although soundness testing was not researched within this project, it is noteworthy because two of the confined mares exhibited edema of the hock, extending down through the fetlock during the majority of this three month research period.

The welfare of PMU mares is guarded by the Recommended Code of Practice for the Care and Handling of Horses on PMU Farms, and by monthly farm inspections (Jones 1996). The welfare of these mares is not dependent upon level of activity as concluded by Clark et al (1991), Hetts et al (1992), or Mal et al (1991). In addition, there is now scientific evidence that PMU mares do not lose physical fitness, according to physiological parameters such as heart rate and blood lactates during their urine collection periods. In conclusion, PMU mares do not appear to have an exercise requirement throughout their urine collection seasons.

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VITA

Celeste Sparks

Candidate for the Degree of

Master of Science

Thesis: INFLUENCE OF RESTRICTED MOVEMENT ON PHYSICAL FITNESS AND THE WELL-BEING OF PREGNANT MARES.

Major Field: Animal Science

Biograhical:

Personal Data: Born in Emporia, Kansas, on November 6, 1971, the daughter of Steven and Marjorie Sparks.

Education: Graduated from Emporia High School, Emporia, Kansas in May 1990; received Bachelor of Science degree in Animal Science from Kansas State University, Manhattan, Kansas in December 1994, respectively. Completed the requirements for the Master of Science degree with a major in Animal Science at Oklahoma State University, July, 1997.

Professional Experiences: Participant in AQHA shows. Veterinary assistant during the last two years of high school, and during summers throughout my junior year in college. Received a research grant from Oklahoma State University made possible by the North American Equine Ranching Information Council.

Professional Memberships: Equine Nutrition and Physiology Society.