THE EFFECTS OF ADDED DIETARY FAT ON ACID-BASE STATUS IN EXERCISING HORSES

by

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INTRODUCTION

In recent years, there has been a large increase in participation in various high performance equine sports, such as polo, three-day eventing, competitive trail-riding, and racing. This has been paralleled by a significant increase in equine research projects concerning cardiovascular fitness, muscle physiology, respiration, and hematology. There has also been considerable interest in nutrition, ergogenic aids, and diet manipulation for the equine athlete.

One of the primary focuses has been that of using high-fat diets to enhance performance of the horse participating in work of varying intensities. Feeding fat has advantages for athletic horses because the diet is energy dense and low in bulk. Adaptation to fatty acid oxidation might spare the use of muscle glycogen, and help delay fatigue. Also, production of carbon dioxide and carbonic acid for a given level of oxidation is less for fatty acids than for carbohydrate. For these reasons, training horses on a high fat diet may help defer fatigue during exercise, which could contribute to improved performance.

The following experiment was conducted to see if a high-fat diet combined with 32 days of interval training had any effects on acid-base status during exercise in young Arabian horses.

LITERATURE REVIEW

ENERGY SOURCES:

Prior to looking at the effects of diet on the exercising horse, it is essential to understand certain aspects of the physiology involved with exercise. Contraction of skeletal muscle for movement requires conversion of chemical energy to mechanical energy. The immediate source of chemical energy in skeletal muscle is adenosine triphosphate (ATP).

ATP is available as an immediate energy source for high intensity exercise of short duration when there is less oxygen available because it is located at the contractile site in the skeletal muscle (Gollnick, 1988). The other means of generating ATP during heavy exercise are by creatine phosphate (CP), the myokinase reaction (MR), and anaerobic glycolysis, all of which take place in the cell cytosol. CP is needed for rephosphorylation of adenosine diphosphate (ADP) to ATP, and the MR involves the conversion of 2 ADP to 1 ATP. These energy sources can only provide the working muscle with ATP only for a very short time, and thus the majority of energy

needed during high intensity exercise is most likely derived from anaerobic glycolysis (Bayly, 1985).

Glycolysis converts carbohydrates (primarily glucose) in the body to pyruvate. The fate of the pyruvate generated depends in part on its concentration, and the amount of oxygen available to the cell. During high intensity exercise oxygen is limited, and the pyruvate concentration rises beyond its capacity for oxidation in the tricarboxylic acid cycle (TCA cycle). The buildup of excess pyruvate from glycolysis is converted to lactate, which is transported to the liver via the bloodstream to be used as substrate for gluconeogenesis. This glucose returns to the working muscle as fuel. In humans, gluconeogenesis can contribute between 6 and 45% of the total glucose output from the liver depending on the intensity and duration of exercise (Ahlborg et al., 1974).

However, using lactate as a substrate is not energetically favored because the production of 1 mol. of glucose from pyruvate requires 6 mol. of ATP. Anaerobic glycolysis is a way to temporarily shift the metabolic burden to the liver, and away from the working muscle (Stryer, 1988). When free glucose is not utilized, it has been shown that sprinting horses can also obtain

glucose for fuel to produce ATP via glycogenolysis in the muscle (Paul, 1971), and the liver (Hultman, 1989), which can proceed under anaerobic or aerobic conditions. Breakdown of muscle glycogen to lactate may be the sole source of energy during heavy exercise, as the uptake of substrate by muscle from blood is not rapid enough to support these conditions. The source of glucose for use during exercise however, ultimately depends on the type and duration of work performed (Gollnick , 1988).

A more efficient means of generating ATP for energy takes place when the supply of oxygen is not limited to any great extent. Aerobic metabolism, or oxidative phosphorylation in the muscle cell takes place in the cytosol and the mitochondria. The pyruvate generated from this aerobic glycolysis is then transported to the mitochondria and converted to acetyl CoA (ACoA). ACoA units enter the TCA cycle for complete oxidation to carbon dioxide (CO_2) and water. The reducing equivalents enter the respiratory chain where ATP is synthesized, with complete oxidation of one mole of glucose yielding 36 ATP. This process requires approximately 5.1 kcal/L of oxygen (Ferrannini, 1988).

Another ATP-yielding process is beta-oxidation of fatty acids. The fatty acids are transported into the

mitochondria by carnitine, with the products entering the TCA cycle as ACoA units for complete oxidation. Oxidation of a typical fatty acid (palmitate) gives a net yield of 131 ATP, and requires less energy per liter of oxygen consumed than does carbohydrate (4.6 kcal/L) (Ferrannini, 1988). These differences are due primarily to the structural differences between carbohydrates and fats, as fats have a larger proportion of carbon and hydrogen.

There are many sources of the substrates utilized during beta-oxidation, which include plasma free fatty acid (FFA) pool, and triglyceride found in the muscle and adipose tissue. It has been shown in man that there is a lag period between the start of exerise and lipolysis in adipose tissue with the subsequent release of FFA for use as an energy source (Pruett, 1970; Pernow and Saltin, 1971). The concentration of intramuscular fat and its contribution as an energy source during exercise is still unclear. Studies with rats (Froberg et al., 1971), and man (Saltin,et al., 1978) demonstrated a decrease in the amount of intramuscular triglyceride with exercise, but other studies have not been able to detect these types of changes (Masoro et al, 1966; Gollnick et al, 1974).

SUBSTRATE UTILIZATION DURING EXERCISE:

The activation of different energy releasing processes during exercise, and the metabolism of a preferred fuel (fat or carbohydrate) for ATP generation depends in part on the intensity, duration, and type of work performed. It has been shown that the greater the work intensity, the greater the breakdown of carbohydrate as fuel (Saltin and Karlsson, 1971). Conversely, preferential fat utilization has been linked by several researchers to long term exercise of submaximal intensity in rats (Rennie et al., 1976; Miller et al., 1984), horses (Pagen et al., 1987), dogs (Hammel et al., 1977; Kronfeld et al., 1977) and man (Pernow and Saltin, 1971; Gollnick et al., 1974; Martin et al., 1978). It has also been shown that the proportion of free fatty acids utilized can increase due to fat feeding alone, by fasting or with training alone (Goodman et al., 1973; Hurley et al., 1986; Pagen et al., 1987).

Fat has advantages as an energy storage form and a metabolic fuel, as it has the highest energy value of any fuel (9.3 Kcal/g), and it can be stored in large amounts throughout the body. However, energy release from fat is a slow process, and requires oxygen, and thus it

cannot be utilized as the sole source of energy except during submaximal exercise. Advantages of utilizing glucose as fuel include the ability to metabolize it aerobically and anaerobically, as well as the fact that glycogen can be the sole source of energy during heavy exercise (Gollnick, 1988). Energy release from carbohydrate is faster than from fat, and because ATP production is related to power output, maximum power can be reached in approximately 3 minutes with glucose oxidation, as opposed to 30 minutes for fat oxidation (Sahlin, 1986). Disadvantages of using carbohydrate as fuel include the requirement of a large volume of water for glycogen storage, which reduces the overall caloric value in the tissue mass. Also, muscle cells depend heavily on their local glycogen stores, and maximal exercise cannot be continued when these are depleted and the muscle cell has to rely on another substrate, such as FFA (Pernow and Saltin, 1971).

There is some evidence that feeding high fat diets can delay exhaustion by supplying the body with energy from fat to spare stored glycogen. Miller et al. (1984) found that after 7 days, rats fed a diet of 78% fat (caloric value) ran significantly longer ($p<.05$) on a treadmill before reaching exhaustion when compared to

rats consuming a control diet. This was in spite of the fact that the rats on the high fat diet had decreased resting levels of stored muscle and liver glycogen. These results suggest an enhanced utilization of the fat, possibly due to muscular adaptation to the diet.

Rennie et. al. (1976) also found a significant sparing of liver and muscle glycogen ($p<.01$) in rats after prolonged moderate exercise following giving corn oil by stomach tube with concomitant administration of heparin subcutaneously to increase free fatty acid levels when compared to control rats. The controls were fed a normal diet, and given an indigestible, unabsorbable cellulose with concomitant adminisrtation of saline subcutaneously prior to exercise. They observed a significant increase in plasma FFA ($p<.01$), and a decrease in the respiratory quotient (RQ) in the corn oil/heparin rats at rest and during exercise, which indicated a shift towards fat utilization. They concluded that this was due to an inhibition of carbohydrate utilization in certain working muscle types, and a slowed depletion of liver glycogen.

Increased levels of plasma FFA were also observed before and after exercise by Hammel et al. (1977) in racing sled dogs fed a diet containing almost no

carbohydrate. Plasma FFA levels were higher in the best performing dogs when compared to the worst performing dogs over a three month period of racing. Training seemed to confer certain advantages to the dogs, such as an increased oxygen carrying capacity due to increased packed cell volume and hemoglobin concentration.

In human subjects, Jansson and Kaijser (1982) showed that feeding a diet high in fat, and low in carbohydrate significantly decreased the use of carbohydrate ($p<.01$) during 25 minutes of submaximal exercise when compared to controls consuming a carbohydrate-rich diet. They also found a smaller muscle glycogen reduction in the fat fed group. They concluded that humans can change the type of fuel utilized for exercise by diet manipulation, and that the fat-rich diet increased the contribution of fatty acids to oxidative metabolism, while sparing glycogen. These results are in agreement with other research using exercising human subjects (Martin et al., 1978), and it has been theorized that fat utilization can be increased by training alone (Hurley et al., 1986) by increasing the levels of enzymes involved with lipolysis.

It is important to mention here that measurements of increased fat oxidation do not always correlate with an

increase in lipolytic rate alone (measured as glycerol appearance), and subsequent FFA release into the circulation through the triglyceride-fatty acid cycle. This cycle plays a major role in fatty acid metabolism in humans undergoing submaximal exercise. Wolfe et. al. (1990) have shown that more than 50% of the increase in fat oxidation could be specifically attributed to the reduction in the rate of re-esterification of fatty acids, and not from increases in the appearance of glycerol alone. At rest, approximately 70% of all fatty acids released during lipolysis were re-esterified, and this rate fell to 25% during the first 30 minutes of exercise, whereas total FA release via triglyceride hydrolysis increased three-fold. Combined with this increased lipolysis at exercise, the cycling greatly enhanced the oxidation of available fatty acids. Whether or not this mechanism is involved with fat utilization in the equine, or how it works during heavy exercise is at present unknown.

PECULIARITIES OF THE EQUINE ATHLETE:

It is necessary to discuss exercise and substrate utilization in the equine separately because of the

unique adaptations to exercise of the respiratory, metabolic, and hematologic systems in the animal. Although similar to other species in limitations of performance due to oxygen transport and utilization in the working muscle, the horse has many peculiarities. Horses can increase oxygen consumption during maximal exercise to a much greater extent than most other species studied (Engelhardt,1977; Pan et al.,1983).

Increased transport of this oxygen is due to a large cardiac output, acheived by a high working heartrate, and a high heart weight to body weight ratio (Snow, 1985). Linked to these advantages is the fact that a significant amount of erythrocytes is stored in the spleen, which are released by splenic contraction during stress, excitement, and exercise, increasing the circulating volume of these cells (Persson et al., 1973).

Some ventilatory responses are also unique to the horse. Traditional theories of ventilatory control in most species during high intensity exercise involve the link between arterial acidosis and hyperpnea. A decrease in pH of arterial blood may stimulate chemoreceptors to produce a hyperventilatory response which results in an arterial hypocapnia to stabilize the respiratory acid-base balance.

Hyperventilation is another response to exercise which may result in part from pulmonary chemo- or mechanoreceptors. However, Pan et al. (1983) found that alveolar ventilation was not highly correlated with the carbon dioxide (CO_2) delivery to the lungs, and that hyperventilation is not mediated by the partial pressure of CO_2 (pCO_2) in the pony. Further studies by Pan et al. (1986) support this evidence, and pointed out that for a given workload applicable over a large range of intensities, the ponies demonstrated a greater hypocapnia than seen in other species. They concluded that the low level of CO_2 in the blood was an advantage to the animal, and contributed to the equine being a superior athlete. These contributions included the maintenance of a normal pH during high intensity exercise, and the ability to maintain the arterial partial pressure of oxygen (pO_2). The exact mechanism for hyperpnea in horses remains unclear.

Another peculiarity that exists in the horse relates to thermoregulation. Heat loss during exercise occurs mainly through evaporation, and one route of this loss is sweating, which is controlled in the equine by the sympathetic nervous system, and the adrenomedullary system (Robertshaw and Taylor, 1969; Snow, 1977). The

horse is similar to man in this respect, however equine sweat is hypertonic, with variable concentrations of sodium ($[Na^+]$), potassium ($[K^+]$), and chloride ($[Cl^-]$). These levels range from 132-249 mmol/L for Na^+, 32-64 mmol/L for K^+, and 165-301 mmol/L for Cl^-(Carlson and Ocen, 1979; Rose et al., 1980; Snow et al., 1982). Equine sweat also contains a higher concentration of protein than many other species of animal (Kerr and Snow, 1983). This is of importance to the high performance horse, as electrolyte and protein losses may be significant during heavy exercise, especially when coupled with improper diet and a high environmental temperature. These factors are important when considering exercise testing programs, and the training of the performance horse.

FAT SUPPLEMENTATION IN THE EQUINE:

Palatability and Digestibility. In order for horses to utilize fat in the diet as a substrate during exercise, the feed must be accepted by the animal, and digested with no unusual gastrointestinal disturbances. The added fat should not affect any other diet components, nor interact with their digestibilities.

Many researchers have investigated the question of which types of fats and oils are most palatable and digestible by the equine. Bowman (1977) conducted preference-style palatability experiments, and digestibility experiments with ponies fed mixed diets containing 0, 10, 20, and 30% corn oil (dry matter basis). Out of the total feed consumed, 52.6% was composed of the basal ration (0% corn oil), 22.9% was the 10% corn oil ration, 14.9% was the 20% corn oil ration, and 9.6% was the 30% corn oil ration. The researchers decided that the 20% corn oil ration would be optimum for use in the digestibility trial because it was high in fat, still palatable, and there were no visible digestive disturbances.

The digestibility trial revealed no significant effects on apparent digestibility (AD) of crude protein (CP), and the AD of fatty acids increased linearly with increasing levels of added corn oil, and was 94% for the 20% corn oil ration. The added fat did not affect serum triglycerides, calcium and magnesium, or blood hematocrit and hemoglobin concentrations.

In addition to studying corn oil, Rich (1980) investigated the palatability and digestibility in the equine of several fats that varied in degree of unsaturation and fatty acid composition. These included

cottonseed, peanut, and safflower oils, 3 animal-vegetable fat blends, and 3 types of tallows (hydrolyzed flakes, inedible, and fancy bleached). Five palatability and 3 preference trials were conducted. When given a choice between fats, corn oil was ranked first in preference for relative palatability, followed by the other oils, the blends, and then the tallows.

In the first of two digestibility experiments performed using the different fats, the AD of acid detergent fiber, the absorption of minerals, and the levels of serum fatty acids, calcium, inorganic phosphorus, magnesium, and glucose were unaffected by any of the added fats when compared to controls. There was a significant increase ($p<.05$) in serum cholesterol in the horses fed the fat diets. In the second digestion trial, true digestibility of the fatty acids showed that all fat sources were similarly digested. The AD of dietary energy showed an increasing trend with all the fats, and significantly increased with corn oil ($p<.05$) when compared to inedible tallow and blend 3.

This is in agreement with McCann et al., (1987), who found that fat supplementation with either corn oil, blended fat, or inedible tallow significantly increased ($p<.05$) dietary metabolic energy when compared to

controls. They also found that the fats produced no differences in heat production as measured by indirect calorimetry, and did not affect protein digestion, or levels of serum calcium, magnesium, and cholesterol. The fats significantly increased ($p<.05$) the energy balance (metabolic energy intake - heat production) by approximately 88% when compared to controls, and contributed to a significant increase ($p<.05$) in apparent fatty acid digestibility. There was also a trend for increased dry matter digestibility.

These results, along with the results of other researchers show that many types of fats and oils are accepted and digested by the equine at high levels in the diet with no visible digestive disturbances. It is also suggested that corn oil has a higher acceptance level by the equine than other sources of fat, as some animal-vegetable blends can result in low palatability and greasy stools (Greiwe, 1990).

Fat Utilization During Exercise. There has been a great deal of research in the past investigating the effects of diet during exercise in horses. An early study (Goodman et al., 1973) showed a significant increase ($p<.05$) in venous and arterial concentrations of plasma FFA in

conditioned horses compared to unconditioned controls. If this increase indicates a better availabilty of FFA, then the authors suggested that the horse may be able to utilize fat during submaximal exercise.

This is in agreement with diet manipulation studies conducted by Hintz et. al. (1978) using horses exercised under prolonged submaximal conditions (endurance riding). The researchers found a significant increase ($p<.001$) in the plasma FFA levels during exercise, but there was no effect of adding an 8% (as fed) feed grade animal fat to the mixed diet. During exercise, muscle glycogen significantly decreased ($p<.01$), and lactate, significantly increased ($p<.05$), but there were no diet effects. There was a significant diet effect ($p<.01$) on plasma glucose concentration. Glucose decreased in both groups with exercise, with the greatest decrease ($p<.01$) in the control horses. The researchers theorized that the partial prevention of a large decrease in blood glucose in the fat horses could be due to the muscle cells preferentially utilizing FFA as a substrate in place of glucose, as has been seen in rats and humans.

A study by Greiwe (1990) employed the use of an equine treadmill to study the responses of horses fed supplemental fat in the form of an animal-vegetable blend

compared to controls. Both groups of horses were exercised 4 days weekly over a 12 week period. The results showed that conditioning decreased blood lactate, serum cholesterol ($p<.01$), and plasma glucose ($p<.001$), and increased plasma FFA ($p<.05$) in both groups at rest and during exercise. There was a diet difference observed in the final week for muscle glycogen levels, which were lower before exercise in the fat horses when compared to the controls, and greater after exercise when compared to controls. This response may indicate a glycogen sparing effect in the horse, but the data indicate that an adaptation period is probably necessary to change over to fat metabolism, as diet differences for muscle glycogen levels were only observed in the final week of the study.

Worth (1988) looked at the effects of a combination of added dietary fat and exercise on AD in the equine. The conditioned horses fed 14% (by weight) added dietary fat in the form of choice white grease had an enhanced AD of CP and dietary energy ($p<.05$) when compared to conditioned horses fed the same basal diet with no added fat.

Based on the many studies reviewed, it seems likely that working horses will readily accept certain

types of added fats in a ration, and that this added fat is highly digestible and may in fact help to enhance the digestibility of certain diet components. It can also be concluded that the added fats studied do not seem to have any detrimental effects on other diet component digestibilities.

INTERVAL TRAINING:

In addition to describing the effects of diet manipulation in the equine, it is important to understand the type of conditioning used in this experiment. The purpose of any training program is to properly condition the athlete in a minimum amount of time without increased exposure to injury. In humans, systematic and intensive methods of training have resulted in a worldwide improvement of track and field records. In the horse racing industry however, improvements in record times have been experienced more often by the Standardbred breed of horses than Thoroughbred horses (Englehardt, 1977). This may be due in part to the fact that Standardbreds are trained more by intermittent exercise periods similar to human interval training programs (Englehardt, 1977). In addition to describing the effects of diet manipulation in the equine, it is important to

understand the type of conditioning used in this experiment.

Humans. Interval training has been used and studied extensively in human athletes, primarily swimmers and runners, with varying results. The two main types of interval training used by humans are aerobic interval training, and anaerobic interval training, sometimes referred to as speed training. Aerobic interval training involves repeated short intervals at an intensity slightly lower than that employed in competition with brief rest intervals of 5-15 seconds (Costill, 1986). The rest periods should allow recovery from muscular strain, without allowing recovery of heartrate or oxygen uptake. Advantages of this type of training are less lactate accumulation in the muscle, less muscular strain, and greater mental stimulation. If these sessions are prolonged, muscle glycogen depletion and increased fat utilization can occur (Wells and Pate, 1988).

Speed training is a very stressful form of interval training, and should be used only sparingly. Short, high-intensity work bouts are employed at maximal or supramaximal levels with rest intervals of approximately 2 minutes in duration. Examples of this in humans are hill running and cycling, which should elicit major

involvement of the anaerobic metabolic processes to increase strength and tolerance of anaerobic metabolism (Wells and Pate, 1988). However, this type of training should only be used after a firm base of muscular fitness has been acheived, as there is increased risk of muscular injury due to the high tensions generated.

Lesmes et. al. (1978) studied two different levels of high-intensity interval training in female runners. One group ran short distances at 170% of VO_2 max either two, or four times per week, with the other group running longer distances at 130% of VO_2 max either two, or four times per week. They found a significant increase ($p<.01$) in VO_2 max, and a significant decrease ($p<.05$) in submaximal heartrate (training induced bradycardia) in both groups. These changes indicated improved fitness, but were independant of the training program. The researchers concluded that the two work intensities were both very high, and could have caused similar stresses on the working muscle, promoting cellular changes of equal magnitude. These results are in agreement with Fox et. al. (1975) who found that improved VO_2 max was not dependent on training level. However, the researchers showed that there was a decreased level of circulatory

stress in subjects training at a higher frequency and duration, as evidenced of a lower submaximal heartrate.

However, these results conflict with those seen in females undergoing interval swim training. McGuigan and Noble (1985) found that both long and short distance interval training programs significantly increased VO_2 max ($p<.05$), but that only short distance intervals produced a significant improvement ($p<.05$) in maximal anaerobic power as determined by the stair running test, and the vertical jump test.

In agreement with these results is a study conducted by Sharp et. al. (1986), who studied cyclists sprint training on a bicycle ergometer compared to endurance trained cyclists. Muscle buffer capacity was calculated with measurements of muscle pH and lactate concentration. Compared to endurance training, the muscle lactate accumulation at exhaustion was significantly increased ($p<.05$) after sprint training, while muscle pH was not affected. This relationship resulted in a significant 37% increase ($p<.05$) in buffering capacity in the sprint-trained cyclists when compared to to their pre-training buffer capacities, and the capacities of the endurance-trained cyclists, which were not significantly different. The reason for this enhanced ability could not be

ascertained, but the researchers suggested that it may be due to an increased protein incorporation in the muscle, or a change in the intracellular bicarbonate and hydrogen ion concentrations. These findings imply that improvement in buffer capacity could be specific for the type of training program utilized. This data, along with other research gives evidence that improved performance may be achieved in certain individuals without extensive or prolonged training sessions if interval-type training is employed.

Horses. Interval training was first employed in horses mainly by Standardbred trainers, who formulated exercise regimens that consisted of a series of short sprints separated by rest periods to allow for partial recovery (Dancer, 1968). A study by Wilson et al. (1987) looked at adaptations of skeletal muscle in thoroughbreds undergoing interval sprint training, and found that repeated bouts of high intensity, short duration work resulted in a significant ($p<.05$) increase in the ratio of type II high oxidative fibers to type II low oxidative fibers. They concluded that the interval training could increase the aerobic capacity of the animal in a fashion similar to that of conventional training regimens. They suggested that if it was possible to improve the

anaerobic capacity in the horse, this would require training the animals to perform maximal sprints lasting no longer than 40 seconds (to stimulate predominantly anaerobic metabolic pathways), and would be difficult to employ without a controlled environment, such as a high-speed treadmill. The researchers stated that horses (especially Thoroughbreds) should be able to improve their performance with sprint training because this breed is genetically adapted to the demands of short bursts of high speed.

In a study by Gabel et al. (1983), interval training was compared to conventional methods in the Standardbred. Although there were no differences between groups in blood lactate level, heartrate, cardiac output, and rectal temperature after exercise, the horses trained by the interval method were able to work 10% faster during sprint sessions. The study also suggested that horses cannot perform as much sprint work (minutes/day) as humans.

Rodiek et al.(1987) did not observe any differences between horses trained by conventional or interval training, but did see an overall significant ($p<.05$) decrease in working heartrate, and a significant increase ($p<.05$) in cardiac output. The researchers pointed out

that a more intense level of interval training may be needed to bring out the differences between training methods. They also stressed the fact that comparisons of methods can be hampered by a lack of understanding concerning which physiological variables are most valuable in estimating fitness in the equine. Variable testing conditons make it difficult to compare results from different studies, and discrepancies in research concerning training effects in the equine point out the need for controlled and reproducible test conditions. One way to help control the variation in test conditions is to employ a repeatable standardized exercise test on a treadmill, which should help minimize the effects of environmental stresses, including weather conditions, footing, rider effects, and track surface.

ACID-BASE STATUS:

Buffering systems. Another aspect of the physiology of exercise can be described by observing the acid-base status of the working animal. All animals must be able to buffer changes within the body to help maintain pH level within a certain range.

The body has many buffering systems, and excluding the mechanisms primarily responsible for urinary

buffering, these systems include: 1) the distribution of hydrogen ions (H^+) between buffer pairs to diminish fluctuations in pH; 2) changing the concentration of extracellular and intracellular bicarbonate and its effects on carbonic acid concentration and the CO_2 for expiration; 3) acceptance of H^+ by plasma proteins such as albumin, which act as weak acids; 4) buffering of the blood through acceptance of H^+ by hemoglobin; and 5) some intracellular buffering by organic phosphate (Madias and Cohen, 1982). When interpretations are made concerning acid-base status, it must be kept in mind that intracellular fluids are not available for direct in vivo measurement, and thus alterations in the extracellular compartments are used to indicate changes in total body content (Gamble, 1982).

Changes in pH alone can classically be described using any acid-base pair. The acid will donate a H^+ and form a base, while a base will accept a H^+ and form an acid. Depending on the relative strength of the acid, there will be differing levels of free H^+, with stronger acids having less binding capacity, and more ions dissociated and available for possible acceptance by the base (Jones, 1987). Consequently, the level of dissociation changes, as does the pH value. Less free H^+

will cause an increase in the pH level, while more free H^+ will cause the pH to decrease. These responses are quantitatively related to the number of H^+ bound by the base (Gamble, 1982).

Another component of the traditional view of acid-base physiology describes the relationship between the regulation of the extracellular hydrogen and bicarbonate ion concentrations in the body, and is represented by the following equation:

$$H^+ + HCO_3^- \text{ <=> } H_2CO_3 \text{ <=> } CO_2 + H_2O$$

Bicarbonate acts as a base by accepting the addition of H^+ to form carbonic acid. The acid can then be broken down to CO_2, which is highly volatile and can be rapidly eliminated from the body through expiration in the lungs. Disturbances in the pCO_2 alone are referred to as respiratory acid-base disturbances. In addition to measuring only pH levels , buffering capacity in the body can also be described in terms of the bicarbonate concentration in the blood, which is often termed non-respiratory, or metabolic acid-base status (Madias and Cohen, 1982). Plasma bicarbonate levels vary with

changes in PCO_2, so metabolic and respiratory disorders are not strictly independent.

Because $[Na^+]$ and $[Cl^-]$ are very high in the extracellular fluid, the buffering reaction with bicarbonate can also be written as the following equation:

$$H^+ + Cl^- + Na^+ + HCO_3^- ==> Na^+ + Cl^- + H_2CO_3 ==> CO_2 + H_2O$$

The H^+ are eventually incorporated into water, but the sodium and chloride remain intact (Gamble, 1982). There is also an intracellular component of buffering that takes place in cells other than the red blood cells. Buffering of intracellular H^+ is not fully elucidated, but may involve exchanges for extracellular Na^+ or K^+, or an acceptance of extracellular bicarbonate in exchange for Cl^- (Thomas, 1984).

Another component which significantly contributes to body buffering is protein, particularly albumin in plasma and hemoglobin in whole blood. Plasma albumin is a weak acid, is not regulated as a component of the acid-base system, and it is therefore an independant variable in the metabolic acid-base balance (Stewart, 1983). Decreases in plasma protein concentration cause

metabolic alkalosis, while increases in plasma protein concentration causes metabolic acidosis (Rossing et al., 1986; Rossing et al., 1988).

Hemoglobin (Hb) is the primary protein buffer in red cells, and the reduced form can buffer more free H^+ than the oxygenated form. When the reduced Hb-H^+ complex reaches the lungs, oxygenation of the Hb generates free (H^+), which combines rapidly with bicarbonate in the presence of carbonic anhydrase. The carbonic acid is ultimately broken down to water and CO_2 which is then released through the lung to help protect the body against acidosis (Jones, 1987).

Under normal conditions in the body, the buffering systems do not show any significant perturbations, and thus maintaining the acid-base balance is accomplished with relative ease. This view is based on assumptions that the pH within the body remains within a very small range. However, this is not always the case during strenuous or exhaustive exercise. The blood pH of working horses has been measured at many levels of exercise, and varies over a large physiological range (Pan et al., 1986; Thornton et al., 1983; Forster et al., 1990). Examination of pH and bicarbonate levels alone is valuable, but may not be the best way to describe acid-

base imbalances that arise from alterations in the concentrations of various electrolytes (Kohn, 1990).

An alternative method of viewing acid-base disorders was introduced by Peter Stewart in 1981, who proposed that the changes in hydrogen and bicarbonate ion concentrations in the body fluids are actually dependent on changes in three independent variables: 1) pCO_2; 2) the concentration of weak acids (mainly proteins), and 3) the strong ion difference (SID). Strong ions are completely dissociated in biological fluids, and the SID is calculated as the difference between the concentrations of positively and negatively charged strong ions in the body fluids. These additional measurements should be important when studying acid-base balance in the working animal. Because of this, many researchers have investigated how exercise affects the different components of acid-base balance, and some have studied the shifts in strong ions and changes in SID during different exercise intensities.

BLOOD GASES AND EXERCISE:

In one of the first studies collecting blood gas data collected during exercise in dogs, Wathen et

al.(1962) studied the effects of various work loads on a treadmill (2.7, 3.6, or 4.4 m/s at an 18% grade) on acid-base balance. They found that in mixed venous blood during exercise, pO_2 decreased, and pCO_2 and $[H^+]$ increased. However, in the arterial blood, pO_2 increased, and pCO_2 and $[H^+]$ decreased with exercise, which resulted in a respiratory alkalosis. They stated that these findings necessitated further investigation into the control of respiration during work, but noted that hyperventilation during recovery after exercise increased the respiratory alkalosis on the arterial side, and caused it to be reflected on the venous side.

Investigation into the acid-base status of horses undergoing different exercise intensities has revealed varying results. Parks and Manohar (1984) looked at acid-base status in ponies working on a treadmill and found, with severe exercise (9 m/s), no change in arterial pO_2, a marked increase in hemoglobin, a significant decrease ($p<.05$) in arterial pH, pCO_2, and bicarbonate, and a significant ($p<.05$) increase in arterial lactate, indicating a marked metabolic acidosis, coupled with a respiratory alkalosis. This was accompanied by a significant decrease ($p<.05$) in mixed venous pH, and pO_2, and a significant increase ($p<.05$)

in pCO_2 . Hemoglobin-oxygen saturation was maintained in part to the mobilization of splenic erythrocytes. The decrease in arterial pCO_2 indicated that the alveolar ventilation in ponies during severe exercise was in excess of that required to remove the increased metabolic CO_2 production, but could not compensate completely for the acidotic state which was aggravated by the increased lactate concentration.

This is in conflict with Milne (1974) who examined blood gas measurements in horses performing different work intensities. Both venous and arterial pH, PCO_2, and bicarbonate concentration significantly decreased ($p<.05$) when compared to resting levels in unconditioned horses undergoing heavy exercise (12 m/sec). A significant increase ($p<.05$) in lactate and packed cell volume (PCV) also occurred. These findings suggested that strenuous exercise in the equine resulted in a metabolic acidosis, coupled with a respiratory alkalosis.

A metabolic and respiratory acidosis was also observed by Bayly et al.(1983) in Thoroughbreds galloping at maximal speed on a racetrack. This response was indicated by a decrease in arterial pH, bicarbonate concentration, and base excess concentration, but there was an increase in arterial pCO_2. They noted that this

increase could hamper the animal's ability to help buffer the changes associated with exercise. The cause of this could be due ventilation-perfusion inequalities, inadequate ventilation possibly due to physical constraints of the lung and chest, or high dead space at rapid respiration rates. Subsequent studies on maximal exercise in Thoroughbreds suggest that mechanical constraints limit any compensatory hyperventilation during this type of exercise (Bayly et al., 1989) but that the horse is able to tolerate the hypercapnia for short periods of time.

These results are in agreement with a study by Thornton et. al.(1983) that investigated the effects of high speed treadmill training on acid-base status in Standardbred trotters. The metabolic acidosis observed during exercise in the animals was due mainly to a large accumulation of lactate, and the respiratory acidosis was seen as the elevated pCO_2, even at five minutes after exercise. These studies reveal the many combinations possibly encountered when studying acid-base balance during exercise, and the measured responses depend a great deal on the intensity and duration of exercise performed

ACID BASE BALANCE AND ELECTROLYTE LEVELS DURING EXERCISE:

While looking at the usual parameters of acid-base status are valuable, there are other factors to consider during high intensity exercise. The energy needed for work that is supplied by glycolysis or oxidation both result in acid end products, or an obligatory production of protons (Mainwood and Renaud, 1984). When there is a high level of lactic acid production, dissociation of this product contributes to changing the $[H^+]$ in plasma and muscle (Kowalchuk et al., 1988). Responses of the muscle cell to an increased acid load (or a reduced pH) also involve several strong ions: K^+, Na^+, and Cl^-, which participate in regulating the $[H^+]$ by several proposed mechanisms: 1) influx of Na^+ in exchange for H^+, requiring no energy; 2) active influx of K^+ in exchange for H^+; 3) efflux of Cl^- in exchange for bicarbonate, which can then combine with intracellular H^+; or 4) active efflux of Cl^- in exchange for a hydroxyl ion, which then combines with intracellular H^+ (Thomas, 1984). There is also evidence that supports the regulation of $[H^+]$ by lactate efflux from the muscle cell (Hultman and Sahlin, 1980).

Because of the potential merit of evaluating the effects of exercise on these ionic differences, Kowalchuk et. al. (1988) studied blood gases, lactate concentration, and the SID in men performing maximal exercise on a bicycle ergometer. The researchers collected muscle biopsies, venous and arterial blood, and monitored intracellular inorganic strong ion changes in the muscle by neutron activation, and in the plasma with ion-selective electrodes. Intracellular lactate concentration increased significantly ($p<.05$) with exercise, accounting for a decrease in intracellular SID (SID_i), which was calculated as $([Na^+] + [K^+] + [Ca^+]) - ([Cl^-] + [lactate])$. Venous pCO_2 and plasma lactate concentration increased, but the plasma SID (SID_e) did not change due to secondary absolute increases in $[K^+]$, $[Ca^+]$, and $[Na^+]$. On the arterial side, there were decreases in pCO_2 and SID_e, and an increase in lactate concentration. The increase in lactate contributed to the decrease in SID_e, and this was reflected in the venous and arterial blood as a decrease in pH.

The researchers also saw a decrease in plasma volume as measured by an increased hemoglobin concentration. They pointed out that the shifts in plasma water could account for some of the changes in the

concentrations of the strong ions in different locations with the exception of potassium. The loss of potassium from the muscle cell resulted in the rise of plasma $[K^+]$. They also concluded that the SID was a valuable measure because it allowed an opportunity to clarify relationships between changes in muscle and plasma, and may help to explain mechanisms that regulate intracellular and extracellular pH during exercise. They added that the SID approach is valid and applicable to many different body systems because it is founded on classic physiochemical principles that can be validated independently by other measurements of $[H^+]$.

These strong ion shifts have been seen in other animals, and Lindinger and Heigenhauser (1988) found a decrease in intracellular $[K^+]$ and magnesium concentration ($[Mg^+]$), and an increase in intracellular $[Na^+]$ and $[Cl^-]$ in the isolated perfused rat hindlimb. These responses were coupled by an increase in plasma $[K^+]$. The researchers concluded that the changes in the concentrations of the strong ions in muscle tissue during intense exercise are the primary factors which contribute to muscle fatigue. This fatigue may be the result of a decrease in glycolytic enzyme activities, due to a disrupted $[H^+]$. An increase in the $[H^+]$ in the muscle

cell may also interfere with the release of calcium for contraction, and changes in SID could contribute to alterations in the membrane potential, which may also be detrimental to contraction. Further studies by the same researchers (Lindinger and Heigenhauser, 1991) support these findings, and suggest that the decrease in muscle $[K^+]$ is the single most important contributor to a decreased membrane potential, and the increased intracellular $[H^+]$. Other researchers have speculated that there is more than one component of fatigue with exercise. Mainwood and Renaud (1985) stated that fatigue in the isolated frog muscle seemed to be due to a combination of intracellular acidosis, which directly affected the myofibrils in the muscle, and a component not dependent on this acidosis. The second component may have been due to a change in the excitation -contraction coupling in the muscle.

Several researchers have investigated the effects of exercise on electrolyte levels in the equine, but evaluation of the SID in horses undergoing maximal exercise is limited. Judson et. al.(1983) examined the effects of sub-maximal and maximal exercise on electrolyte levels in conditioned Thoroughbreds. Maximal exercise (16 m/sec) resulted in a significant increase

($p<.05$) in plasma [K^+], no change in [Cl^-], and a significant increase ($p<.001$) in plasma sodium and albumin. These and other changes elicited during maximal exercise were more severe than at sub-maximal intensity, and these changes may be informative when assesing the fitness of horses.

These responses are in agreement with Harris and Snow (1988), who studied the effects of high intensity (12 m/sec) exercise on lactate and electrolyte levels using Thoroughbreds running on a treadmill. Plasma [K^+], [Na^+], protein, and lactate concentrations increased with exercise, but there was no consistent change in [Cl^-]. They concluded that the rise in plasma [K^+] was dependent on the exercise intensity, and did vary between horses. The results seemed to support the idea that this change may also affect muscular contraction in horses at high work intensities. The accumulation of lactate and H^+ may slow the re-uptake of K^+ released during muscular contraction, possibly due to an inhibition of the Na^+-K^+ pump in the muscle.

Although there are few studies that have evaluated SID in exercising horses, based on results from studies measuring ion fluxes, it seems that electrolyte changes in horses are similar to responses found in other

species. Evaluation of acid-base status in the equine should include an examination of the SID, to help better characterize the movement of ions between the interior of the muscle cell, extra-cellular fluid, red blood cells and plasma, irregardless of the fact that the exact quantitative origins and mechanisms of transport can only be speculated.

ACID-BASE BALANCE, EXERCISE, AND FAT SUPPLEMENTATION:

The effects of high fat diets on acid-base status have not been extensively investigated. Measurements made by indirect calorimetry highlight the basic differences in the structure and utilization of either fat or carbohydrate. Complete oxidation of 1 mol of glucose gives a net yield of 36 mol of ATP, uses 134 L of oxygen, and produces 134 L of carbon dioxide, giving a respiratory quotient (RQ) of 1. Complete oxidation of 1 mol of fatty acid (palmitate) yields 131 mol of ATP, uses 515 L of oxygen, and produces 358 L of carbon dioxide, giving an RQ of 0.695 (Ferrannini, 1988). Because fat oxidation also produces less CO_2 per kcal of substrate than carbohydrate (CHO) (0.043 L CO_2 for fat; 0.199 L CO_2

for CHO), high fat diets may help to reduce the CO_2 load in the blood in this fashion.

Early studies in humans have examined the RQ during different work intensities, and have demonstrated the shifts from carbohydrate utilization with heavy exercise, to the utilization of fat during low and moderate intensities (Pernow and Saltin, 1971; Jansson and Kaijser, 1982). These types of studies are much more difficult in the horse because of limitations in measuring gas exchange during exercise with a mask and flowmeter apparatus.

Pagen et al., (1987) employed a 3 X 3 Latin Square design to measure the effects of diet on RQ in Standardbred horses at different exercise intensities. A stepwise test was performed on an equine treadmill which consisted of 2 minute increments of work at 2, 4, 6, 8, and 10 m/sec. Results showed that the RQs during exercise were significantly higher ($p<.05$) when the horses were fed a control diet, as compared to diets high in fat (15% fat in the form of soybean oil) or protein (20% crude protein). A low intensity, long distance exercise test was also studied, which consisted of speeds of 5 m/sec for 105 minutes with rest periods every 15 minutes. These results revealed a significant increase

($p<.05$) in RQ for all groups during the first 18 minutes of exercise when compared to resting values, and a significant decrease ($p<.05$) in the RQs of the high fat and protein horses at 63, and 93 minutes of exercise when compared to the control horses. These findings show that horses can utilize different substrates at different work intensities, and also that the horse is able to shift from one fuel to another during long distance exercise. Horses seem to be able to preferentially use carbohydrate at higher workloads, and shift to fat utilization during long term, low intensity exercise, in a manner similar to findings in humans.

These findings are evidence for support of diet manipulation in the equine athlete when looking at preferential fuel use. Whether or not high-fat diets can confer any advantages to the animal during heavy exercise is not well established, but there are recent findings that support this idea. Webb et al. (1987) found an improvement in the anaerobic performance of cutting horses when fed supplemental fat as judged by a cutting test to exhaustion.

It is also unknown if acid-base balance, particularly CO_2 production and subsequent acid load, can be altered during exercise with high-fat diets, and if

this change is even measureable in the venous blood. If it is possible to change these components and measure the differences, it is unknown exactly how animal performance may be improved, and how this improvement can be objectively evaluated.

OBJECTIVES

Enhancing the performance of horses by feeding a high level of dietary fat has been previously investigated. High levels of energy are required by the equine athlete, and can be met by feeding a large amount of carbohydrate (grain) in the diet. However, this practice may lead to azoturia, laminitis, and colic in the horse. Therefore, many types of fats and oils have been investigated as more concentrated forms of energy for exercising horses.

The metabolism of high fat diets results in less carbon dioxide per liter of oxygen consumed when compared to carbohydrate, and is therefore less acidogenic. This may help confer an advantage to the animal by delaying fatigue due to acidosis during exercise of high intensities. The present study investigated the use of corn oil as a high fat dietary supplement in the horse, combined with high intensity interval training over a period of 15 weeks. The acid-base status of exercising horses consuming a high fat diet was compared to the acid-base status of exercising horses consuming a control diet. The effects of the 15 week training period on both groups were also investigated.

EXPERIMENTAL PROCEDURE

Eight arabian horses (5 fillies and 3 geldings) between 36 and 48 months of age were used in this trial. All horses were accustomed to working on a high speed treadmill while simultaneously having blood samples obtained from catheters in the neck. Horses were housed in a 60 m x 78 m dirt paddock during the day, and brought into 3.7 m x 2.3 m loose box stalls bedded with sawdust from 4 pm to 9 am. The stalls were not located in the barn that housed the treadmill. Horses had free access to water at all times. A loose granular vitamin-mineral mixture[1] was available free choice in the paddock (Appendix Table 1).

Horses were groomed on exercise days, and any minor cuts were treated topically with Nitrofurazone dressing. All horses were dewormed with ivermectin[2] 3 weeks after the start of exercise, and feet were trimmed at 6 and 12 weeks after the start of exercise.

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[1] Equi-Choice, Wilson Enterprises Inc., Disputanta, VA 23842

[2] Eqvalan, MSDAGVET, Merck and Co., Inc., Rahway, NJ 07065

The control diet (C) consisted of chopped hay, cracked corn, molasses, and a vitamin premix[3]. The high fat diet (F) was similar to the control except cracked corn was replaced by corn oil at a level of 10% by weight. Rations were balanced for nutritive value to meet or exceed the NRC (1989) requirements for heavy work in young horses. Analysis of the diets is given in Tables 1 and 2, and the vitamin premix contained 429,000 IU/kg of vitamin A, 176,000 IU/kg of vitamin D, and 15,994 IU/kg of vitamin E. Feed samples were obtained monthly for analysis to ensure uniformity during the trial. Horses were fed the diets at approximately 2% of body weight (as fed) per day in two equal portions at 7 am and 4 pm. Horses were all fed the control during a 3 week acclimation period prior to the baseline Standard Exercise Test (SET). Horses were weighed weekly on a large animal scale[4] to monitor weight changes and ensure proper growth. Feed consumption was adjusted to maintain a body condition score between 5 and 7 with no excess amount of food refused. Body condition scores and explanations are shown in Appendix Table 2.

Horses were paired according to endurance ability observed in previous exercise tests (see SET protocol),

[3] Wilson Enterprises Inc., Disputanta, VA 23842

[4] EZ-Weigh, Dyco, Inc., Cave Creek, AZ 85331

TABLE 1. INGREDIENT COMPOSITION OF DIETS

	CONTROL		FAT	
ITEM (%)	AS FED	100% DM	AS FED	100%DM
Orchardgrass hay, sun-cured, mid-bloom	48.0	43.2	48.0	43.2
Molasses, sugarcane	8.0	5.9	8.0	5.9
Cracked corn grain	43.0	37.8	30.0	26.4
Limestone	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5
Corn oil[a]	0	0	10.0	10.0
44% Soybean meal	0	0	3.0	2.7

[a] donated by Corn Products Corp., Best Foods Unit, Union, NJ

TABLE 2. CHEMICAL COMPOSITION OF DIETS

Item	Control		Fat	
	100% DM	As Fed	100% DM	As Fed
Dry matter, %	89.3	---	90.1	---
Composition of dry matter, %				
Crude protein	10.0	8.9	9.3	8.4
Digestible protein	5.8	5.2	5.1	4.6
Ether extract	2.3	---	15.7	---
Acid detergent fiber	31.8	28.4	34.0	30.6

and one horse of each pair was randomly assigned either the control (C) or high fat diet (F), with the other horse of the pair receiving the opposite diet. The fat diet was introduced gradually over a period of five days to avoid any digestive disturbances. Prior to receiving these diets, all horses underwent the baseline SET in a climate-controlled barn (T:55-60° F; Hum:30-50%) on a high speed equine treadmill[5]. The protocol for the SET is shown in Table 3. The SETs were performed on two consecutive days, with 2 F and 2 C horses running each day. Horses were worked until they reached exhaustion, which is defined for this experiment in Table 4.

Prior to the morning of the SET, food was witheld from the horses for at least 12 hours. Resting heartrates were obtained in the stalls with a stethescope, and rectal temperatures[6] were also taken at this time. Horses to be tested that day were moved to holding stalls in the treadmill barn at least 2 hours prior to the start of their exercise test. Resting heartrates and rectal temperatures were taken again after acclimation to the surroundings. Heartrates were monitored during exercise using a digital commercial

[5] Mustang 2200, Kagra Ag, 5615 Fahrwangen, Switzerland

[6] Omron Marshall Products, Inc., Lincolnshire, IL 60069

TABLE 3. STANDARD EXERCISE TEST PROTOCOL

Treadmill speed (m/s)	Time (min)[a]	Slope (%)
Warm-up: 1.3	3	0
1.3	3	6
Stepwise test: 2.3, 2.6, 3.0, 3.4, 4.0, 4.4, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0	3	6

[a] Treadmill speeds during stepwise test were increased at 3 min. intervals

TABLE 4. DEFINITIONS OF EXHAUSTION

Sudden uneveness in stride
Uncoordination in gait
Excessive stumbling and tripping
Refusal to move forward off the back rope
Cannot be urged forward by pulling on the leadrope

receiver and electrodes[7] held in place by a surcingle. At least one hour prior to the start of exercise, all horses had heparinized[8] 14g x 5.5 in venous catheters[9] with 5 ml, 89 cm extension sets[10] implanted in the left jugular vein. Resting blood samples were taken at a standstill on the treadmill, with the remaining samples taken just prior to each speed increase. One sample of blood was collected anaerobically into a 3 cc heparinized plastic syringe[11], capped to prevent exposure to air, and placed in an ice-water bath. This blood was analyzed in duplicate within two hours for pH, and pCO2 at 37°C on a blood gas analyzer[12]. Rectal temperatures were monitored during exercise whenever possible to adjust the blood gas measurements to body temperature. Bicarbonate and actual base excess were calculated from this data using nomogram equations (Siggaard-Andersen, 1963).

Another blood sample was collected in a plastic 35 cc syringe[11]. An aliquot of whole blood was frozen for

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[7] Kagra Ag, 5615 Fahrwangen, Switzerland

[8] Elkins-Sinn, Inc., Cherry Hill, NJ 08034

[9] Abbocath-T, Abbot Hospitals, Inc., North Chicago, IL 60064

[10] Baxter Healthcare Corp., Deerfield, IL 60015

[11] Monoject, Sherwood Medical, St. Louis, MO 63103

[12] B.M.S. Radiometer, 2400 Copenhagen NV Denmark

determination of hemoglobin content[13]. Whole blood was not collected during SET 1 and consequently, the average hemoglobin values from SETs 2 and 3 were used for these values. An aliquot of whole blood was precipitated in 8% perchloric acid (1:2), centrifuged, and the supernatent drawn off and frozen at -70°C. Analysis for lactate[14] was done within 5 days. The remaining blood was placed in tubes containing lithium heparin[15], immediately centrifuged, and the plasma drawn off and stored at -70°C. Plasma was analyzed in duplicate within 10 days for chloride[16] and albumin[17]. These procedures were performed using an autopipettor[18] and a spectrophotometric analyzer[19]. Plasma samples at rest

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[13] Proc. 525, Sigma Diagnostics, St. Louis, MO 63178

[14] Proc. 826-UV, Sigma Diagnostics, St. Louis, MO 63178

[15] Vacutainer, Becton Dickinson, Rutherford, NJ 07070

[16] Proc. 461, Sigma Diagnostics, St. Louis, MO 63178

[17] Proc. 631, Sigma Diagnostics, St. Louis, MO 63178

[18] Pipettor 2000, Baker Instruments Corp., Allentown, PA 18103

[19] Centrifichem System 500, Union Carbide Corp., Pleasantville, NY 10570

and exhaustion were analyzed by potentiometry[20] for Na^+ and K^+ within 2 months of the SET. The strong ion difference was calculated as: ($[Na^+]$ +$[K^+]$) - ($[Cl^-]$ + [lactate]) (Stewart, 1981) at rest and exhaustion only. The SET was repeated after 16 days of exercise (interval #1) and again after another 16 days of exercise (interval #2). Data were analyzed using the General Linear Models Procedure for analysis of variance with repeated measures[21]. Due to the fact that no horses were consuming the fat diet at the first SET, data were also analyzed using the first SET as a covariate. Any interactions with a significance level of $p<.05$ was subjected to follow-up analysis using Tukey's studentized range test (SAS, 1985). Wherever non-parametric analysis was appropriate, the Wilcoxon sign-rank test was used (Ott, 1988).

The daily exercise protocol consisted of exercise 4 days per week (4 days work alternated with 3 days rest) according to the schedule shown in Table 5. The speeds used were chosen to be estimates of 70% of average maximum speed (trots) and 80% of average maximum speed (gallops). Resting heartrates, and initial and final

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[20] Ektachem, Eastman Kodak Co., Rochester, NY 14650

[21] SAS, SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC

TABLE 5. DAILY EXERCISE PROTOCOL

Speed(m/s)	Gait	Slope(%)	Time(min)
	Interval #1		
1.6	walk	0	5
3.2	trot	6	4
7.0	gallop	6	2
4.8	trot	6	4
8.0	gallop	6	2
4.8	trot	6	4
1.6	walk	0	3
	Interval #2		
1.6	walk	0	5
3.2	trot	6	4
7.0	gallop	6	2
4.8	trot	6	4
8.0	gallop	6	2
4.8	trot	6	4
9.0	gallop	6	2
4.8	trot	6	4
1.6	walk	0	3

rectal temperatures were monitored on all exercise days. Heartrates were recorded prior to each change in speed. Horses were evaluated for lameness at each gait during exercise according to the scoring system in Table 6. Any horse that missed daily treadmill exercise made these days up the following week so that all horses had the same total number of days of exercise between SETs. These horses were also administered phenylbutazone (1-2g) orally, but no medication was given for at least 72 hours prior to any of the SETs.

TABLE 6. LAMENESS SCORES

Score	Explanation	Exercise
0	No visible lameness signs	Yes
1	Slightly irregular gait and discomfort	Yes
2	Inconsistent signs of mild lameness	Yes
3	Persistent signs of moderate lameness	No
4	Moderate to severe lameness	No
5	Severe lameness	No

RESULTS AND DISCUSSION

One week after the baseline SET, one of the fillies in the fat group was removed from the study due to a sudden difficulty in breathing attributed to a suspected laryngeal hemiplagia. The rest of the SETs were conducted using the remaining 7 horses. Three weeks after the baseline SET, one filly in the control group exhibited signs of myositis, and was administered 50 mg of fluxinine meglumide[22] IV, and 40 mg of vitamin E/selenium[23] IM. Two weeks after SET 2, one of the geldings in the control group exhibited signs of colic, and was administered dioctyl-sodium sulfosuccinate via nasogastric tube, and phenylbutazone IV.

FEED CONSUMPTION, BODY WEIGHTS, AND TIME TO EXHAUSTION:

Feed consumption data are listed in Table 7, and shows that both the fat and control horses ate significantly less ($p<.05$) feed in the period prior to

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[22] Banamine, Schering Corp., 10409 I Street, Omaha, NE 68127

[23] BO-Se (1 mg/ml), Schering Corp., 10409 I Street, Omaha, NE 68127

TABLE 7. AVERAGE DAILY FEED INTAKE

	Control[a]	Fat[b]
SET #	kg	
1	8.5	8.0
2	8.4	7.9
3	7.4[c]	7.0[c]

[a]Mean of 4 animals
[b]Mean of 3 animals
[c]Means with superscripts differ significantly ($p<.05$)

SET 3 as compared to the other time periods. There were no significant differences between groups for feed intake. Estimated energy intakes are shown in Table 8. The digestible energy (DE) of the fat diet per kg was higher than the control diet, and the DE intake per day for the fat horses was 20% higher than the control. Average bodyweights are shown in Table 9, and show that even though the horses on the fat diet consumed less feed during the period prior to SET 3, they weighed significantly more ($p<.05$) at SET 3 when compared to SETs 1 and 2. There were no significant differences in the control group over time, and no significant differences between groups. Several studies have shown a decrease in feed consumption and no loss in bodyweight with high fat diets, indicating efficient utilization (Hintz et al., 1978; Duren et al., 1987; Meyers et al., 1987).

Data on time to reach exhaustion can be seen in Table 10, and shows that both groups took significantly longer ($p<.05$) to reach exhaustion by SET 3 when compared to SETs 1 and 2, but there were no significant differences between groups. This is in agreement with results from a study by Greiwe (1990), and is one general indication of an improvement in fitness level. It was originally hoped that this measurement would help distinguish between the two diets for fitness level, with

TABLE 8. ESTIMATED AVERAGE ENERGY INTAKE[a]

	Control[b]	Fat[c]
Avg. daily feed intake (kg/d)	8.10	7.60
Digestible energy, est. (Mcal/kg)	2.4	3.1
DE intake/d (Mcal)	19.44	23.56

[a]Calculated on 100% dry matter basis
[b]Mean of 4 animals
[c]Mean of 3 animals

TABLE 9. AVERAGE BODYWEIGHTS

SET #	Control[a]	Fat[b]
	kg	
1	425	402[c]
2	425	399[c]
3	434	413[d]

[a] Mean of 4 animals
[b] Mean of 3 animals
[cd] Means in the same column with different superscripts differ significantly ($p<.05$)

TABLE 10. AVERAGE TIME TO REACH EXHAUSTION

	Control[a]	Fat[b]
SET #	min	
1	42.5[c]	41.0[c]
2	44.8[c]	46.0
3	50.8[d]	49.0[d]

[a]Mean of 4 animals
[b]Mean of 3 animals
[cd]Means in the same column with different superscripts differ significantly ($p<.05$)

the fitter group taking longer to reach exhaustion. Evaluation of exhaustion was as objective as possible, but caution must be taken when making conclusions about fitness level based on this appraisal because it is still very subjective. The remaining results reported are least squares means (LSM), (control: n = 4; fat: n = 3) for analysis of variance using SET 1 as a covariate unless otherwise specified, and a summary of numerical LSM is shown in Appendix Table 3.

HEARTRATES:

Responses of heartrate to exercise can be seen in Figure 1. There was a significant overall increase ($p<.001$) in heartrate over time, but there were no significant diet effects. The effects of training on the mean resting heartrate during the SETs can be seen in Figure 2. Non-parametric analysis revealed a significant 7% reduction in resting heartrate by SET 3 ($p<.009$) when compared to SET 2, but no significant diet differences.

The effects of training on mean submaximal working heartrate can be seen in Figure 3. Non-parametric analysis showed significant overall decreases in heartrate ($p<.009$) between SETs 1 and 2 , and SETs 2 and 3 at 20 minutes of exercise. This time was chosen for analysis because all horses were exercising at a fast

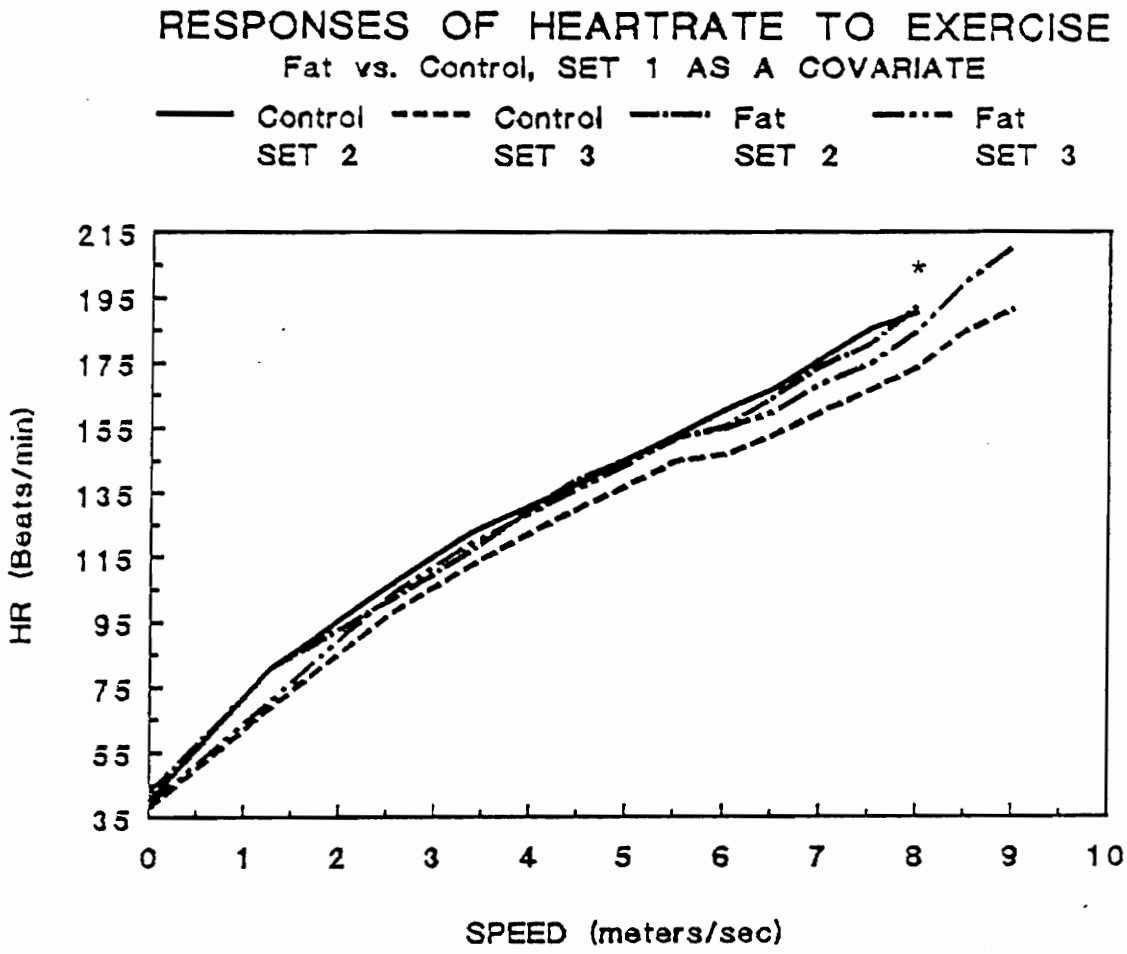

Figure 1. Responses of heartrate to exercise and 15 weeks of training: control diet vs. fat diet (SE: control : ± 0.989; fat : ± 1.142)
* denotes significance ($p<.001$).

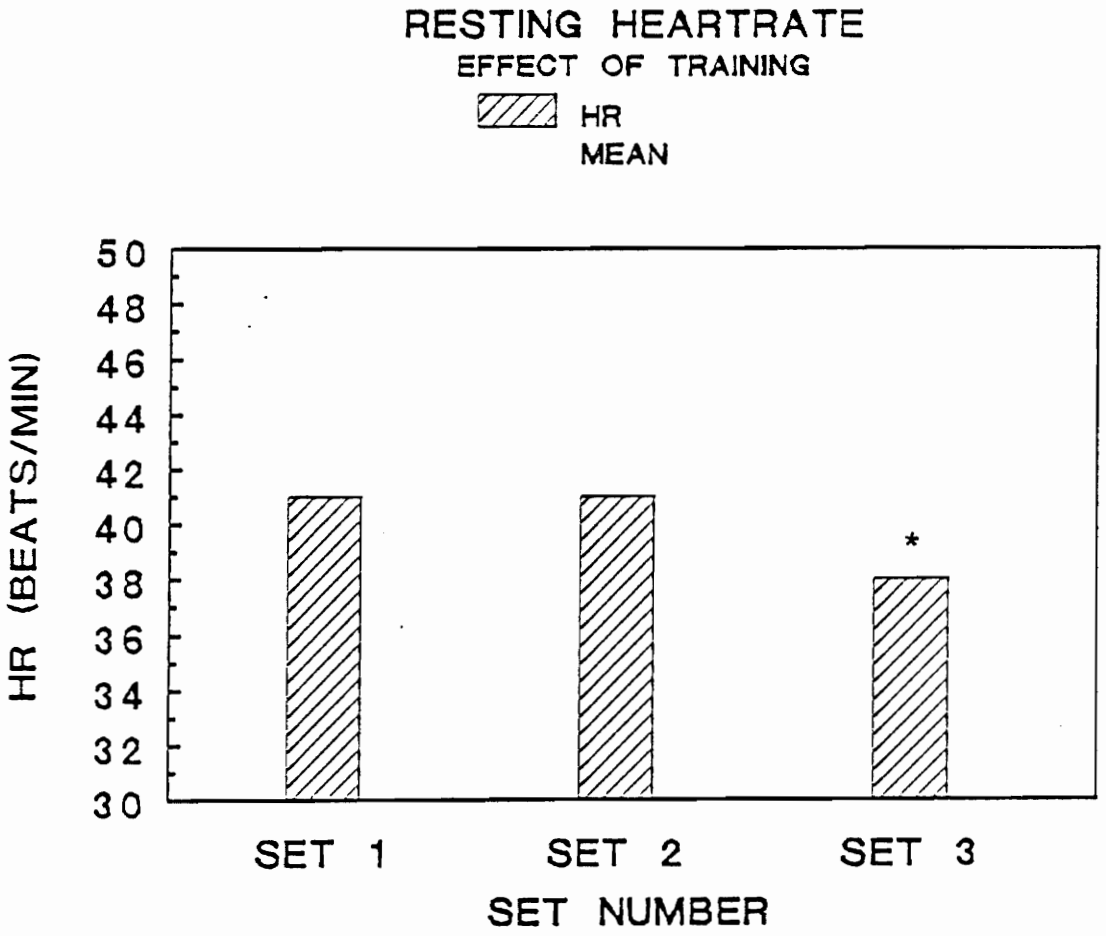

Figure 2. Effects of 15 weeks of training on mean overall resting heartrate (SE: ± 3.53; n = 7) * denotes significant difference ($p<.009$).

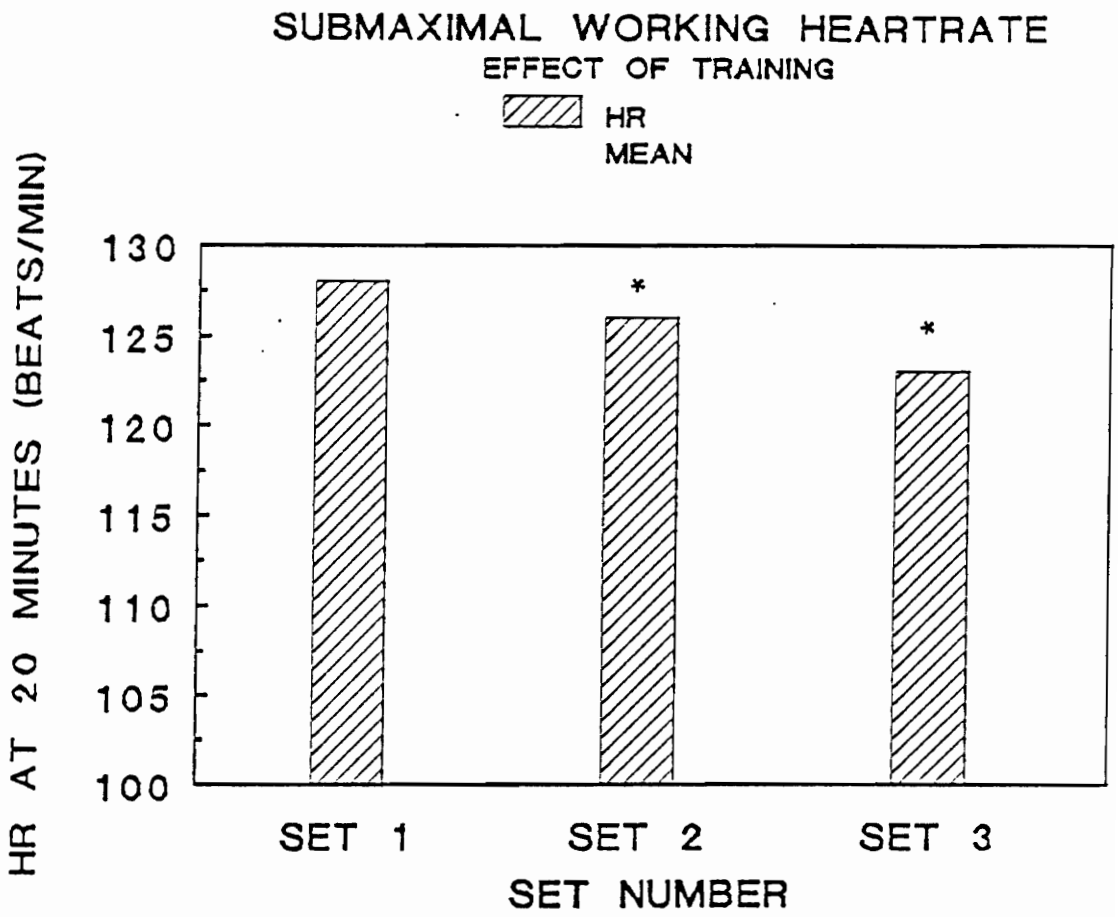

Figure 3. Effects of 15 weeks of training on mean overall submaximal working heartrate (SE: ± 1.17; n = 7) * denotes significant difference ($p<.009$).

trot at this speed (4.4 m/sec), and seemed to be entering a steady state of exercise. However, there were no significant diet differences. These results conflict with those reported by other researchers, who found that fat diets resulted in lower working heartrates when compared to conventional diets (Pagen et al., 1986).

Maximum heartrates reached during the SETs ranged from 180-212 bpm, and maximum heartrates acheived during daily sprints ranged from 205-235 bpm (9m/sec). The effects of training on overall mean maximum heartrate (at exhaustion) during the SETs can be seen in Figure 4. Non-parametric analysis revealed significant increases ($p<.009$) in heartrate at exhaustion between SETs 1 and 2 (12% increase), and SETs 2 and 3 (5% increase). This indicated that the horses were able to exercise at higher maximal heartrates over the 15 week training period, and is a general indication of improved fitness (Thomas et al., 1983). During daily exercise, it can be assumed that the horses were working near maximal intensity during sprints, as peak heartrate in the horse is generally believed to be 220-240 bpm (Engelhardt, 1977). Daily exercise and resting heartrates did show a decreasing trend over the 15 week study, but it was not significant.

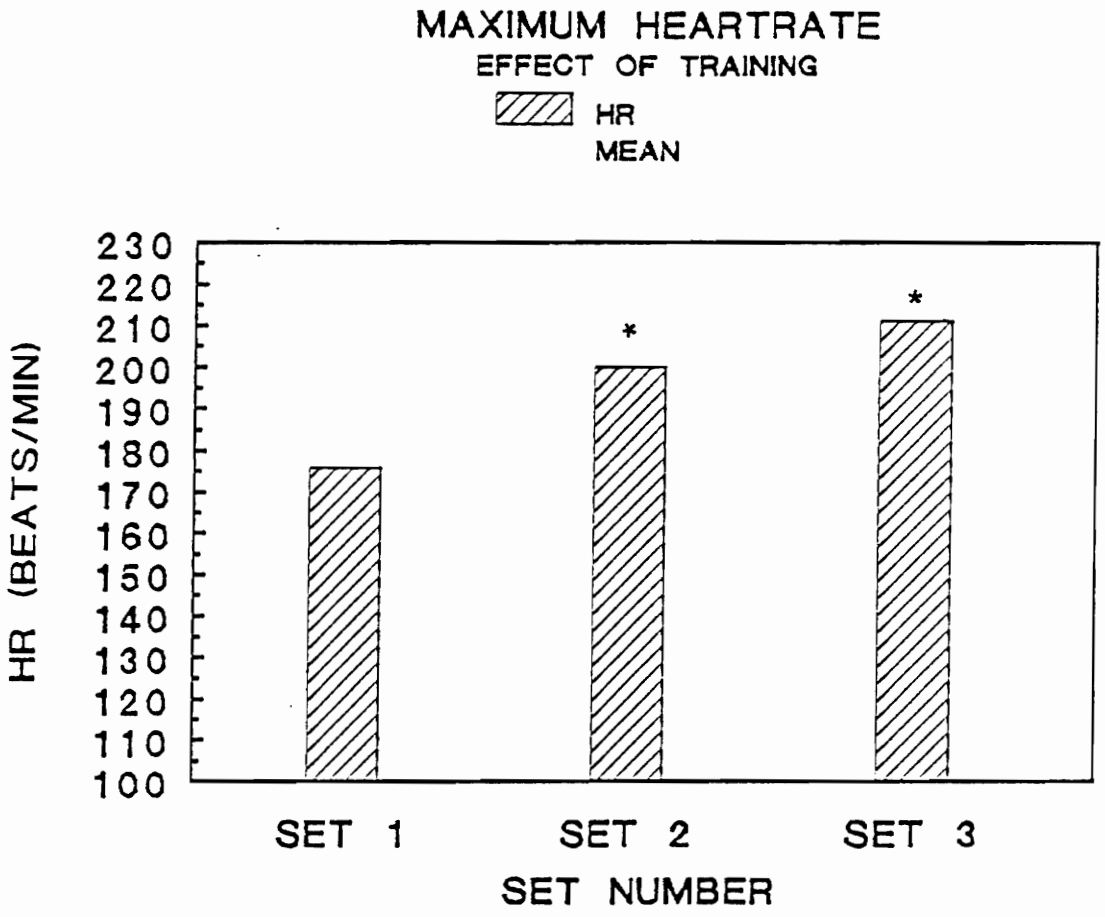

Figure 4. Effects of training on mean overall heartrate at exhaustion (SE: ± 1.52; n = 7)
* denotes significant difference (p<.009).

WHOLE BLOOD HEMOGLOBIN AND PLASMA ALBUMIN:

Results for the changes in hemoglobin over time can be seen in Figure 5. This data was obtained only during SETs 2 and 3, and therefore were not analyzed using SET 1 as a covariate. The data show a significant increase ($p<.05$) in blood hemoglobin with exercise (23% average increase), but no significant diet differences. There was a slight training effect, which can be seen in Figure 6, as there was an overall 4% increase ($p<.10$) in blood hemoglobin at SET 3 when compared to SET 2. The increase in hemoglobin during exercise is due to erythrocyte release from splenic contraction (Persson et al., 1973). This increase is an advantage to the animal, as it allows a greater oxygen-carrying capacity in the blood (Parks and Manohar, 1984), and may increase as much as 60% in the horse, as the storage capacity of the equine spleen is much greater than other species (Engelhardt, 1977). The slight training effect suggests the possibility of improvements in oxygen-carrying capacity with training over time. Enhancement of this ability may confer further advantages to the animal during heavy exercise, but the frequency of observance of this response to training in horses is unknown.

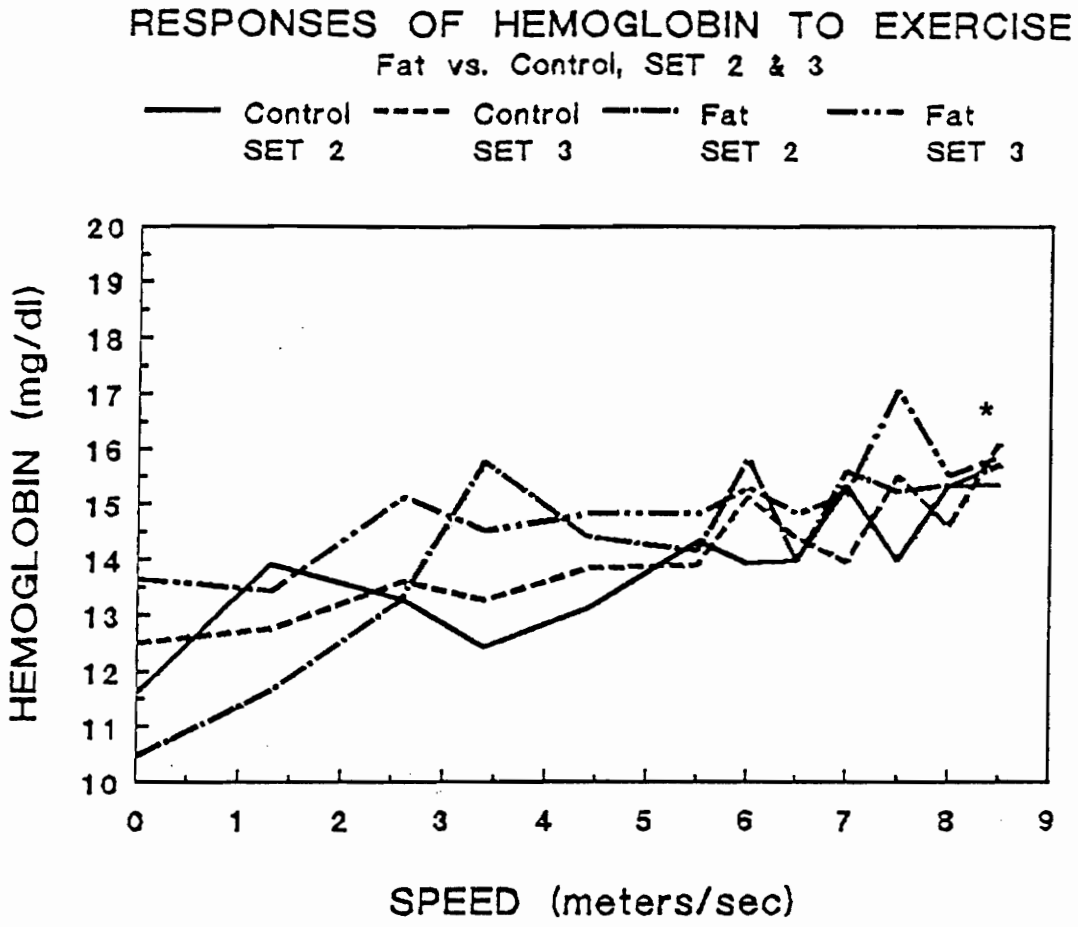

Figure 5. Responses of whole blood hemoglobin to exercise and 15 weeks of training: control diet vs. fat diet (SE: control : ± 0.836; fat : ± 0.966) * denotes significance ($p<.01$).

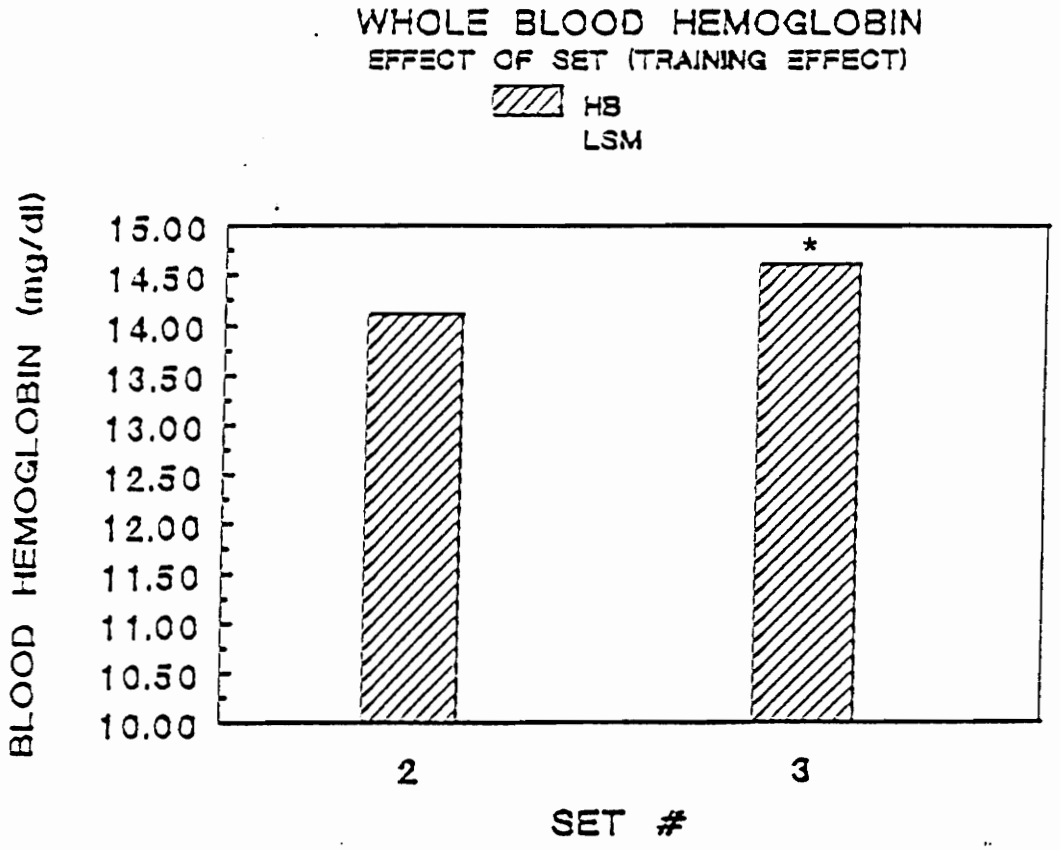

Figure 6. Effect of training on overall whole blood hemoglobin (SE: $\pm$ 0.683) * denotes significant difference ($p<.05$).

Responses of plasma albumin to exercise can be seen in Figure 7. Although there was an average 8% increase with exercise, it was not significant, there were no significant training effects, and there were no significant differences between diets. Increases in plasma albumin are generally attributed to dehydration, and can be an indicator of increased plasma solids. However, because the SETs were performed in a climate-controlled barn, sweating was only moderate, even during high intensity exercise, and may have helped to minimize water losses and hemoconcentration.

BLOOD pH AND PCO_2:

Responses of pH to exercise can be seen in Figure 8. There was a significant decrease ($p<.001$) in pH over time, but there were no significant differences between groups. Figure 9 illustrates that there was a significant interaction ($p<.05$) between diet and SET because the pH of the control horses at SET 2 was significantly greater than any other value. This may suggest that the control horses were better able to buffer the acidosis at SET 2, or they produced less acids at this time, but the difference was lost by SET 3.

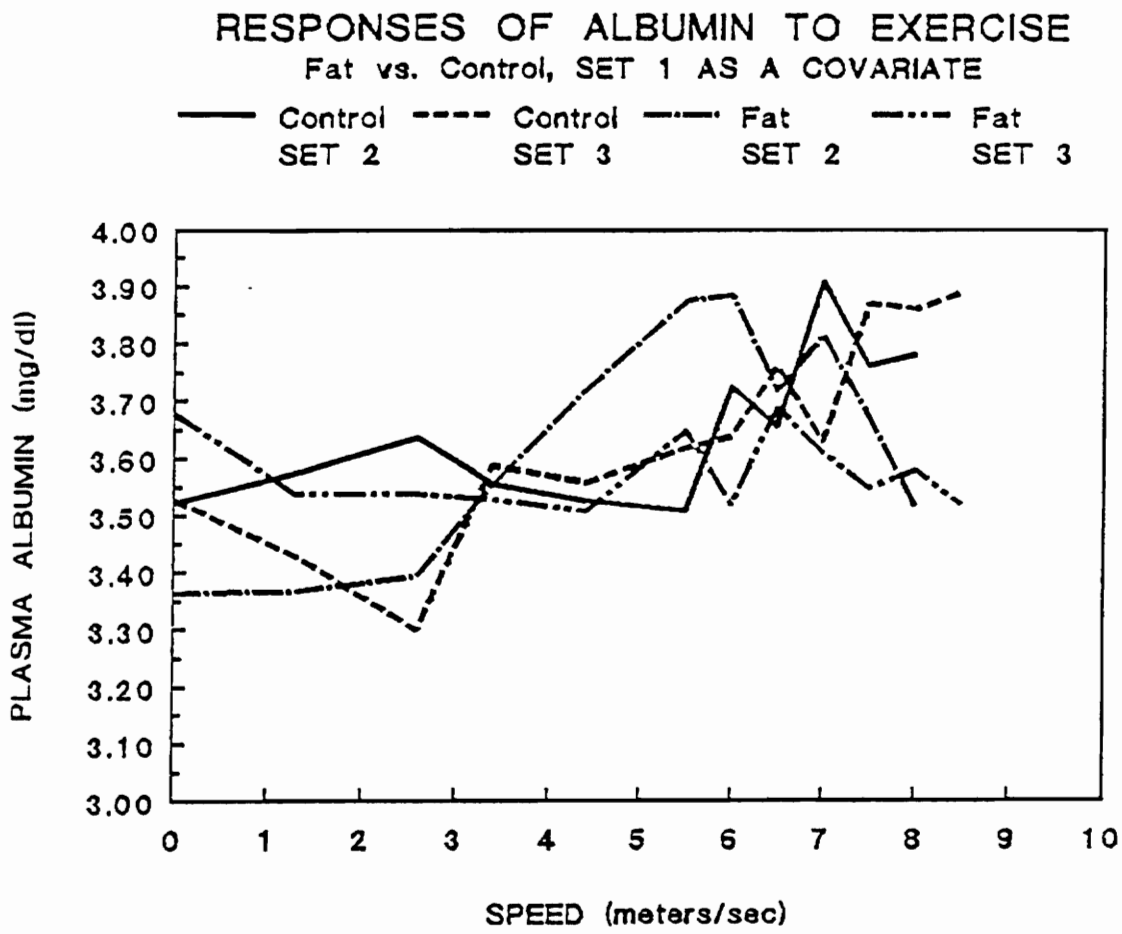

Figure 7. Responses of plasma albumin to exercise and 15 weeks of training: control diet vs. fat diet (SE: control: ± 0.184; fat: ± 0.213)

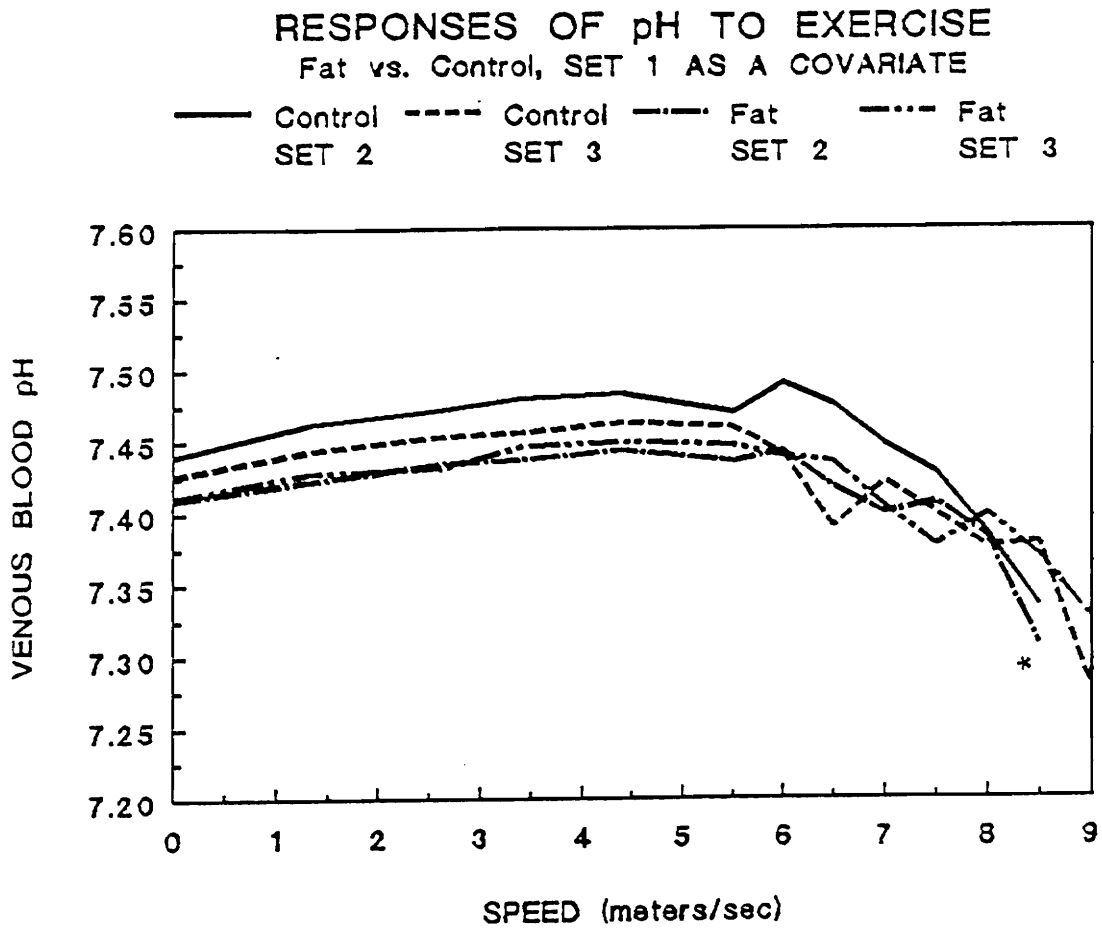

Figure 8. Responses of blood pH to exercise and 15 weeks of training: control diet vs. fat diet (SE: control : ± 0.018961; fat : ± 0.021968)
* denotes significance (p<.001).

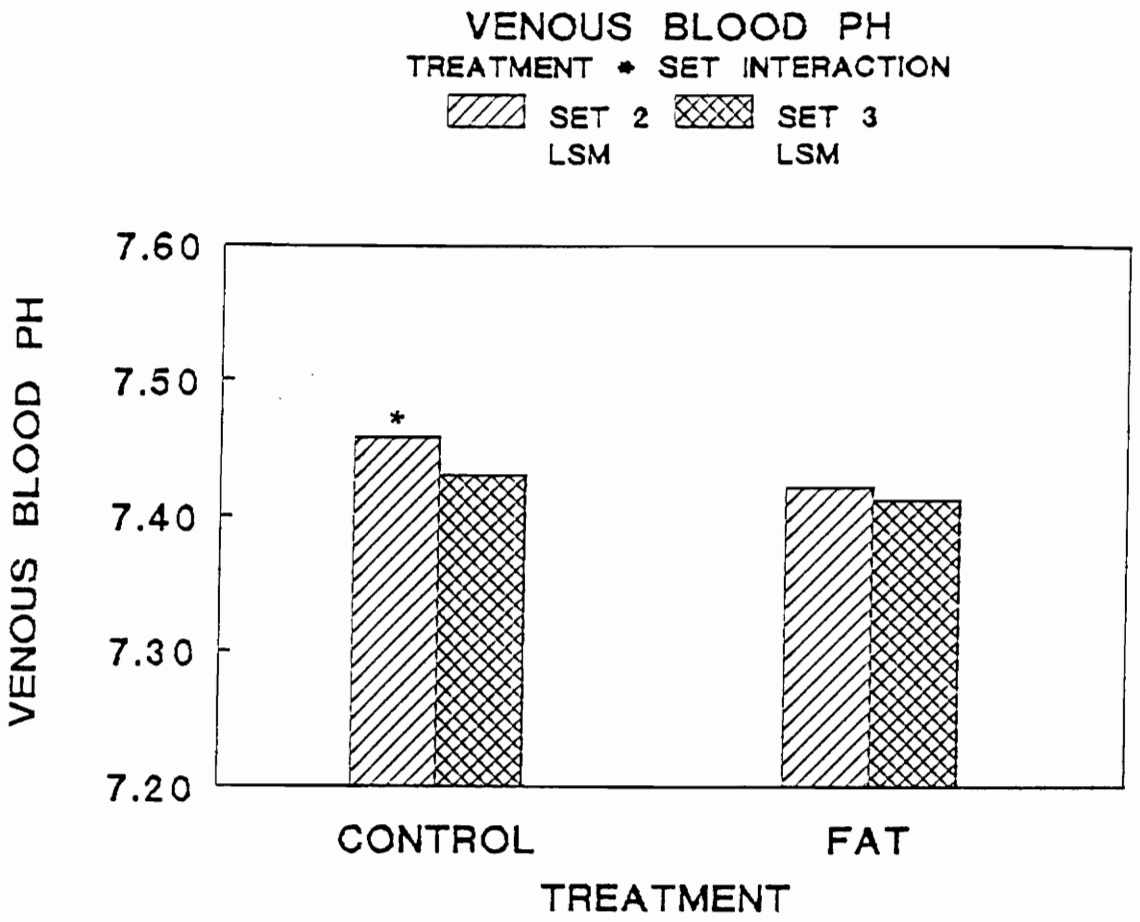

Figure 9. Diet*SET interaction for overall blood pH (SE: control : ± 0.03771; fat ± 0.03898) * denotes significant difference ($p<.05$).

Responses of pCO_2 to exercise can be seen in Figure 10. There was a significant decrease ($p<.001$) in pCO_2 over time, indicating a respiratory alkalosis, but there were no significant differences between diets. There was a slight training effect overall ($p<.10$), most likely due to the overall 12% decrease in the pCO_2 of the fat group at SET 3 when compared to SET 2. This probably is due to an increase in hyperventilation, but may suggest that the high fat diet combined with training helped decrease pCO_2 in the blood of the fat group.

This can be seen more clearly when looking at the significant diet and SET interaction ($p<.01$), which indicated that the fat group had a 5% higher level of CO_2 in the blood at SET 2 when compared to SET 3 (Figure 11), and that this difference was slightly greater (7%) when compared to either control group. There were no differences between any groups by SET 3, so training may have helped the horses in the fat group to decrease the pCO_2. The higher overall pCO_2 in the fat group does not reflect the advantages potentially offered by the fat diet, mainly less CO_2 production, and thus less acid load on the body systems. Two explanations that can be offered are: 1) the higher caloric density of the fat diet increased the overall CO_2 production in this group of horses. The fat group did not maintain a constant

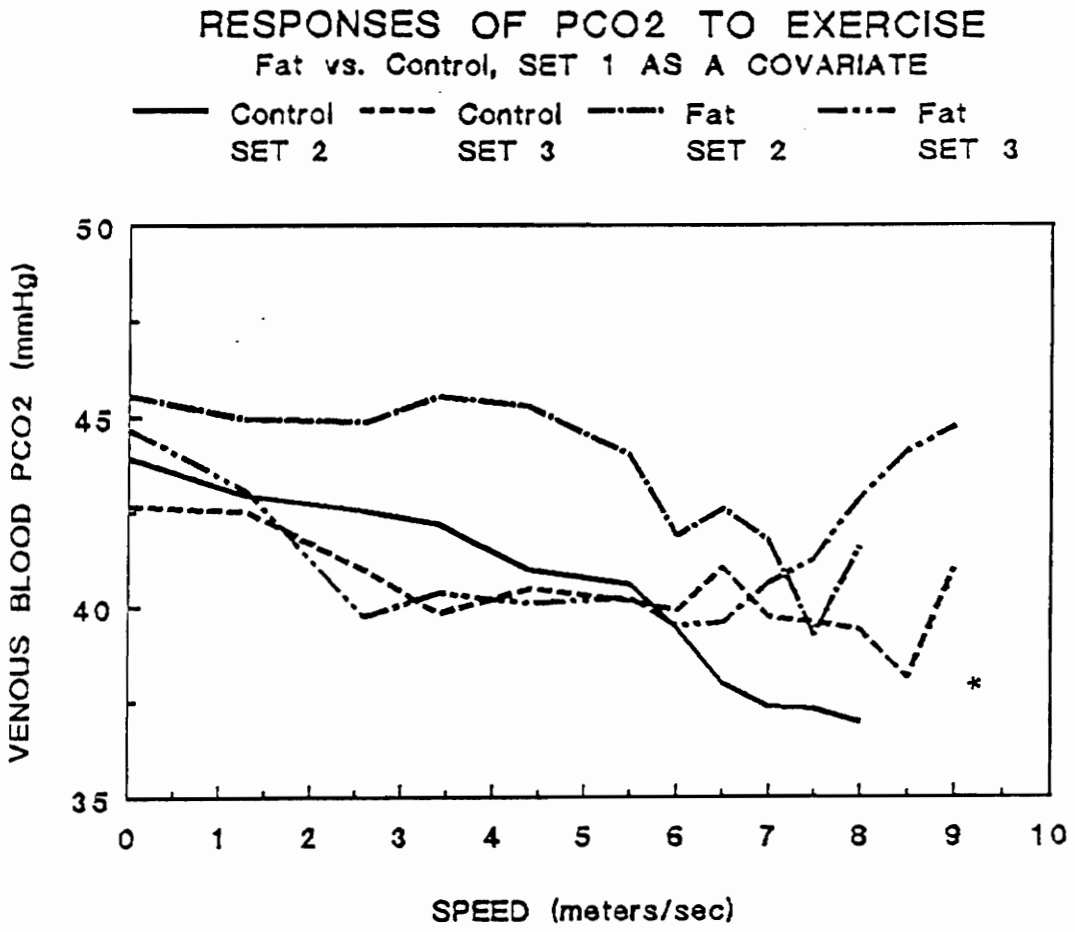

Figure 10. Responses of pCO_2 to exercise and 15 weeks of training: control diet vs. fat diet (SE: control : ± 1.562; fat : ± 1.805) * denotes significance ($p<.001$)

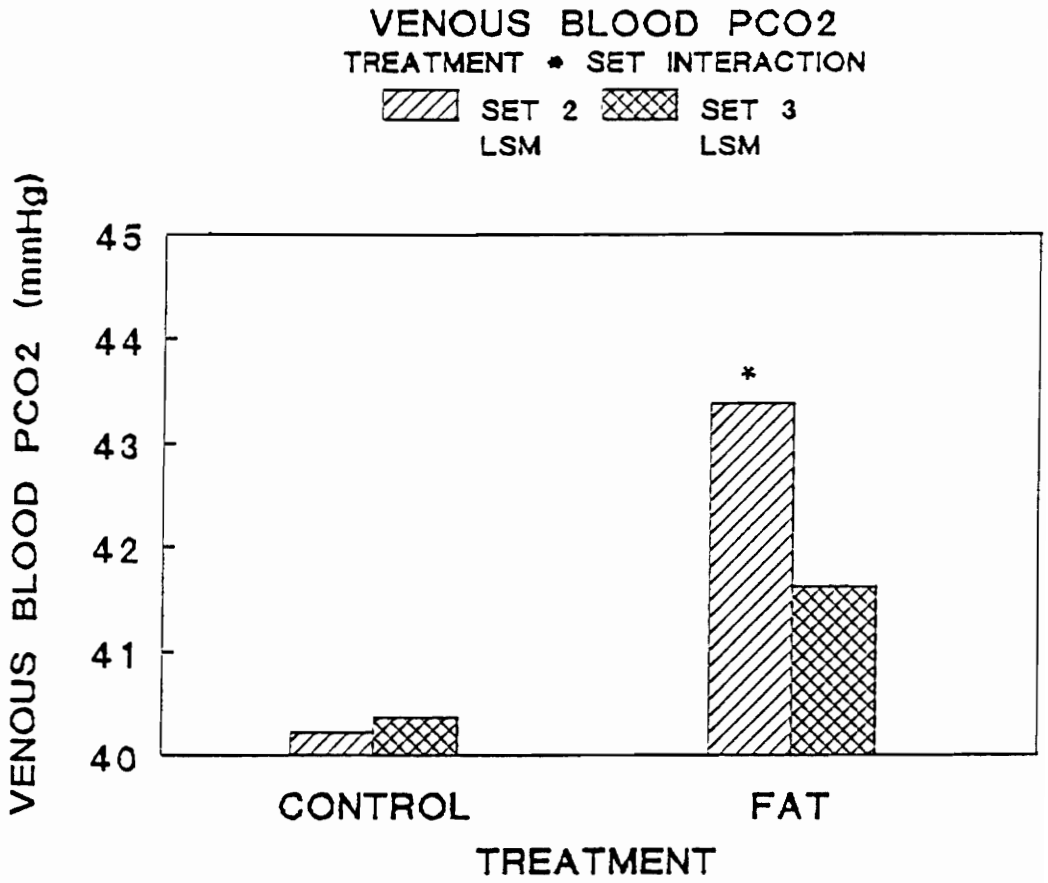

Figure 11. Diet*SET interaction for venous pCO_2 (SE: control : $\pm$ 2.227; fat : $\pm$ 3.128) * denotes significant difference.

body weight throughout the trial, but gained weight in the period prior to SET #3. It is also possible that 2) the sampling site chosen (jugular vein) was not the best indicator of gas exchange in the muscle, as the blood had already traveled to the lung, heart, and head before it was sampled. A mixed venous sample may offer a better estimate of muscle metabolism, with collection of direct muscle effluent being the best indicator of muscle gas exchange. Also, it may be possible to decrease any calorigenic effects of the increased diet density by ensuring that the diets fed are isocaloric.

CALCULATED BLOOD BICARBONATE AND ACTUAL BASE EXCESS:

Results for the effects of exercise on calculated plasma bicarbonate are shown in Figure 12. There was a significant average 18% decrease ($p<.001$) in bicarbonate levels over time, indicating a "metabolic acidosis", but there were no significant differences between groups. Figure 13 illustrates that there was a significant training effect ($p<.05$) which indicated that the level of bicarbonate in the blood was lower overall by SET 3. One explanation may be that the slight increase in hemoglobin with training, combined with a high blood flow rate to the lung has caused the overall reduction in bicarbonate

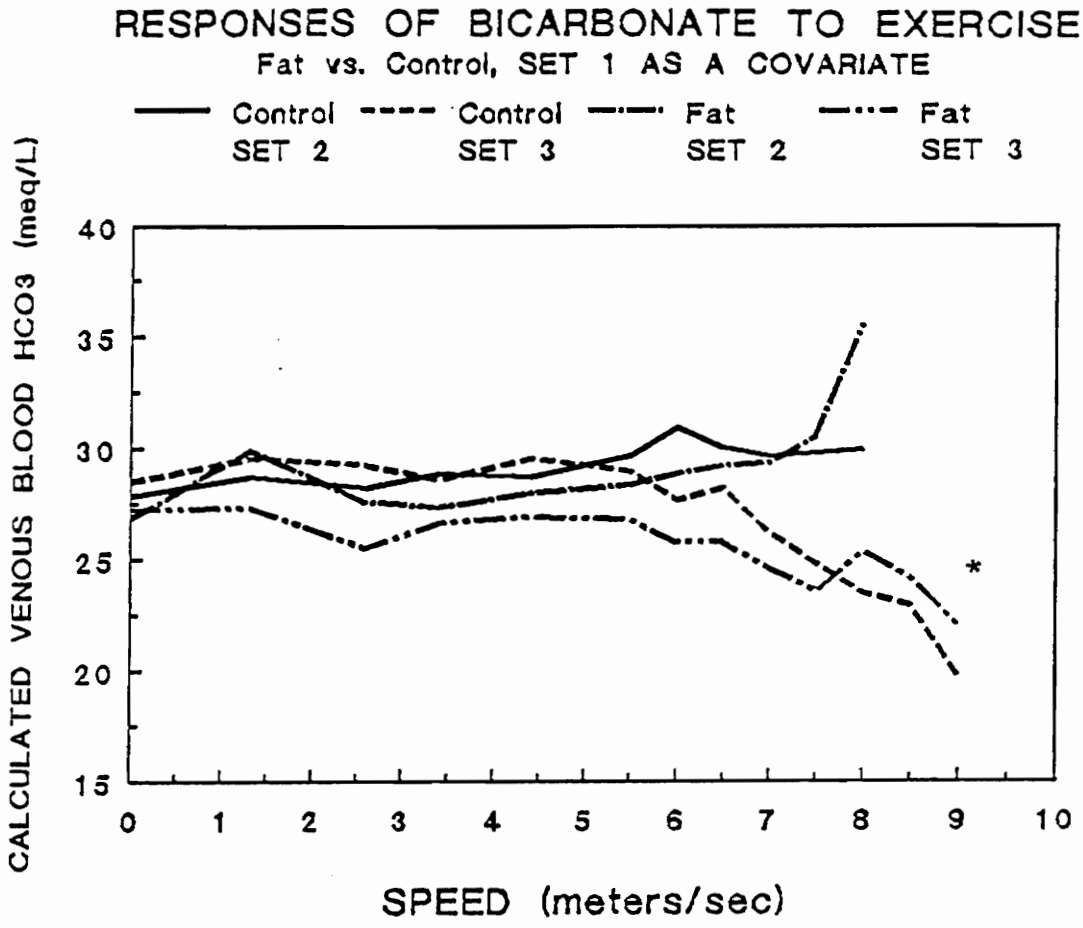

Figure 12. Responses of plasma bicarbonate to exercise and 15 weeks of training: control diet vs. fat diet (SE: control: ± 1.17; fat: ± 1.37)
* denotes significance ($p<.001$)

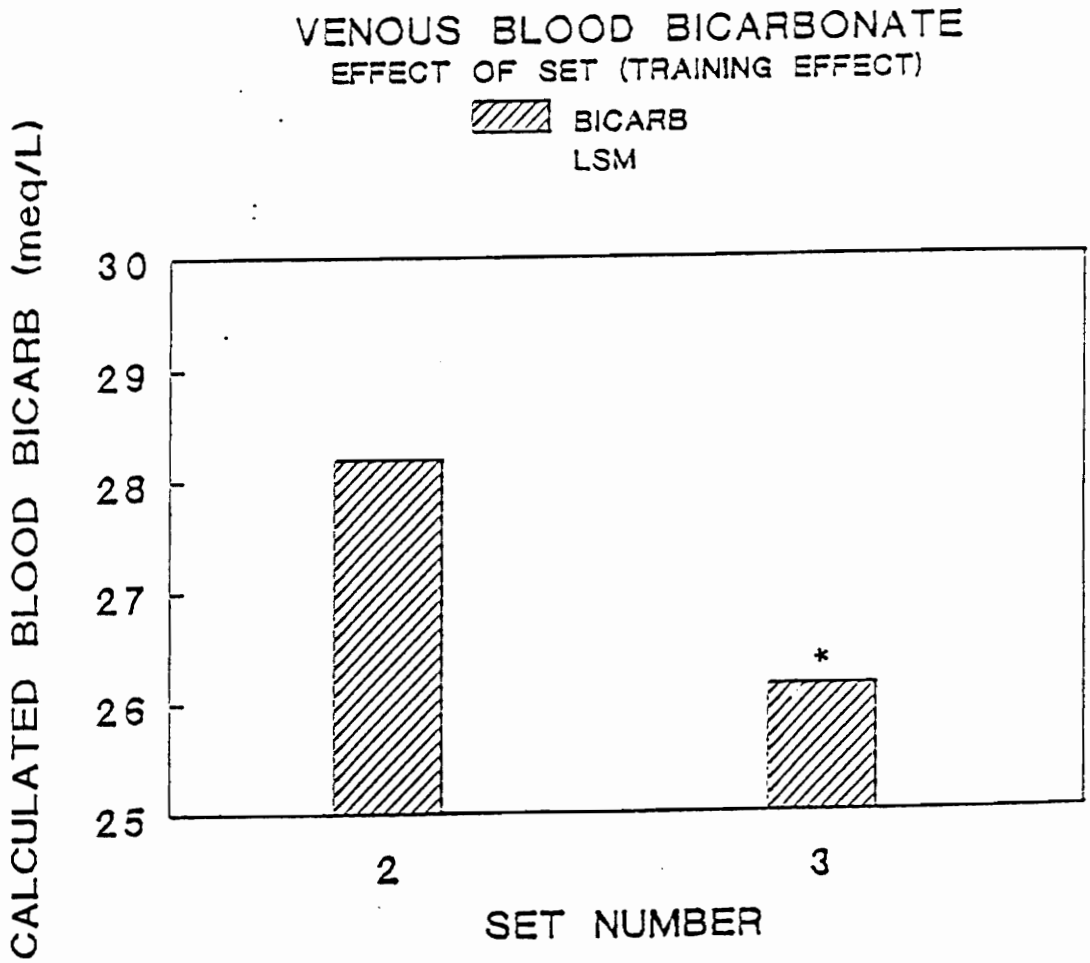

Figure 13. Effects of training on overall plasma bicarbonate levels (SE: ± 1.07) * denotes significant difference ($p<.05$).

in the plasma. When the erythrocytes reach the lung after traveling from the working muscle, it is possible that bicarbonate in the red cell must pick up the excess H^+ which was removed from the hemoglobin in exchange for oxygen, and push the equilibrium of the buffering equation towards production of CO_2 and water in the cell. There could then be less bicarbonate, and more CO_2 in the cell to cross the membrane into the plasma. It is difficult to say, however, whether this is what is actually happening, and it is important to remember that bicarbonate is a calculated value for the plasma only. The responses of bicarbonate to exercise are dependent on the pCO_2, and the pH of the blood.

Responses of calculated actual base excess to exercise are shown in Figure 14. There was a significant overall 12% decrease ($p<.001$) in base excess over time, but there were no significant differences between groups. The base excess eventually became negative, and indicates an overwhelming of the buffering systems by acid, possibly lactate. There was a significant training effect ($p<.001$), as there was an overall 54% decrease in the base excess by SET 3, shown by Figure 15. This decrease indicates excess acid load as well, but takes into account the hemoglobin in the red cells. There was also a significant ($p<.05$) treatment * SET interaction

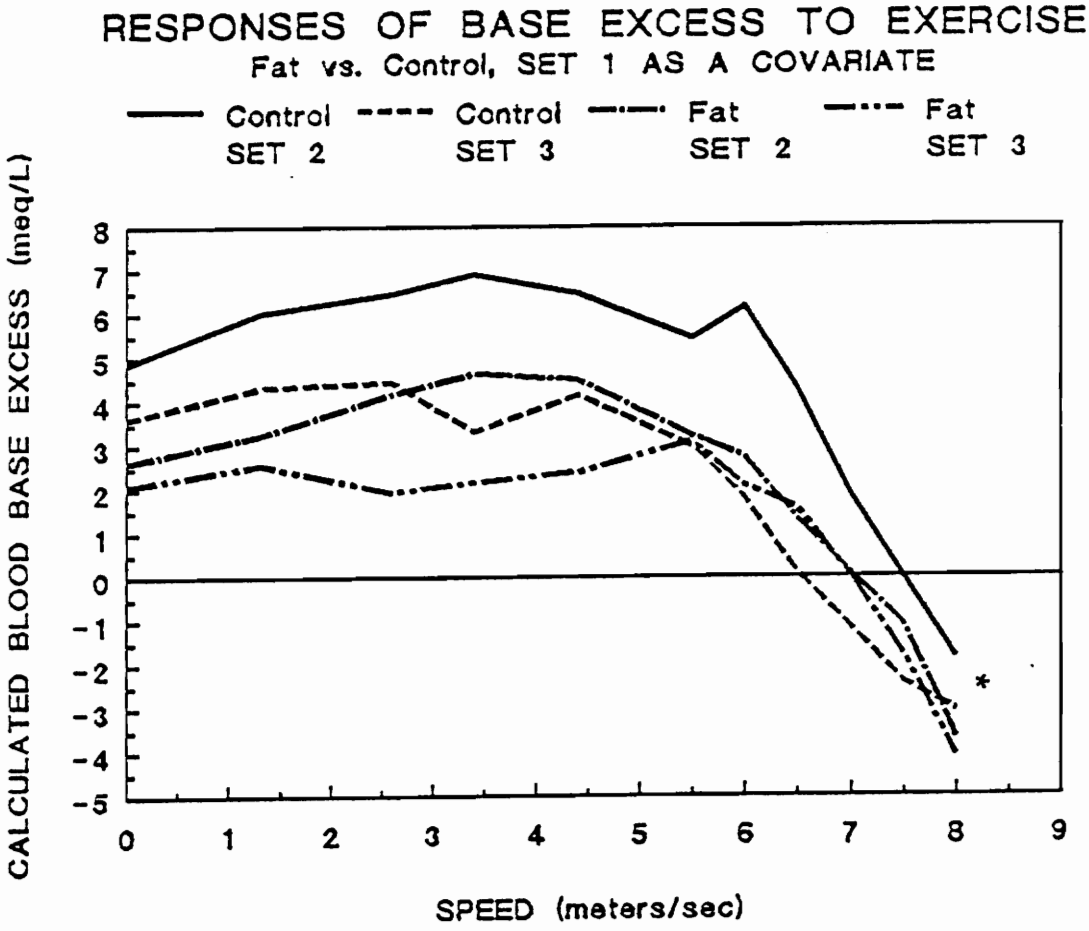

Figure 14. Responses of base excess to exercise and 15 weeks of training: control diet vs. fat diet (SE: control ± 1.44; fat : ± 1.46) * denotes significance.

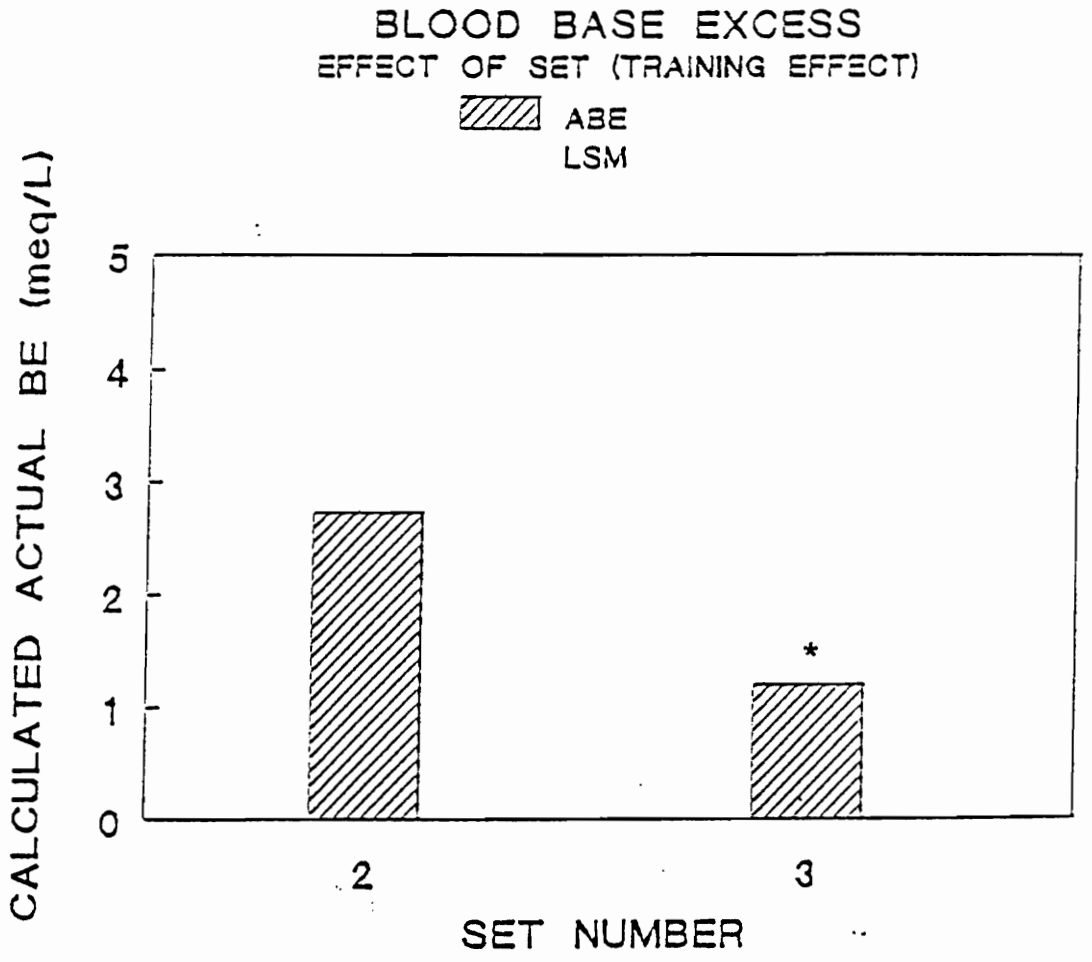

Figure 15. Effects of training on base excess (SE: $\pm$ 1.26) * denotes significant difference ($p<.05$).

for base excess, as shown by Figure 16. By SET 3, the fat group shows a significant ($p<.05$) negative base excess compared to all the other values, and is an 87% difference when compared to the control group in SET 2. This large negative value illustrates the excess acids in the blood of the fat group at SET 3, and may be due to a larger accumulation of lactate in the blood of this group. This may actually be beneficial to the working animal, as an increase in blood lactate may indicate an enhanced removal from the muscle. It seems that this advantage may be enhanced by the fat diet combined with training.

The base excess is a calculated measurement, and is a valid way to give a general idea of the deviation from normal whole-blood buffer base. However, it is important to realize that this calculation alone is not always accurate, and should only be used for evaluation of acid-base status in addition to other measurements because it incorrectly assumes that the carbon dioxide titration curve in vitro and in vivo are equal (Harrington et al., 1982). It is important to remember that base excess is also a dependent variable, and can change with perturbations in blood gases, pH, and hemoglobin concentration.

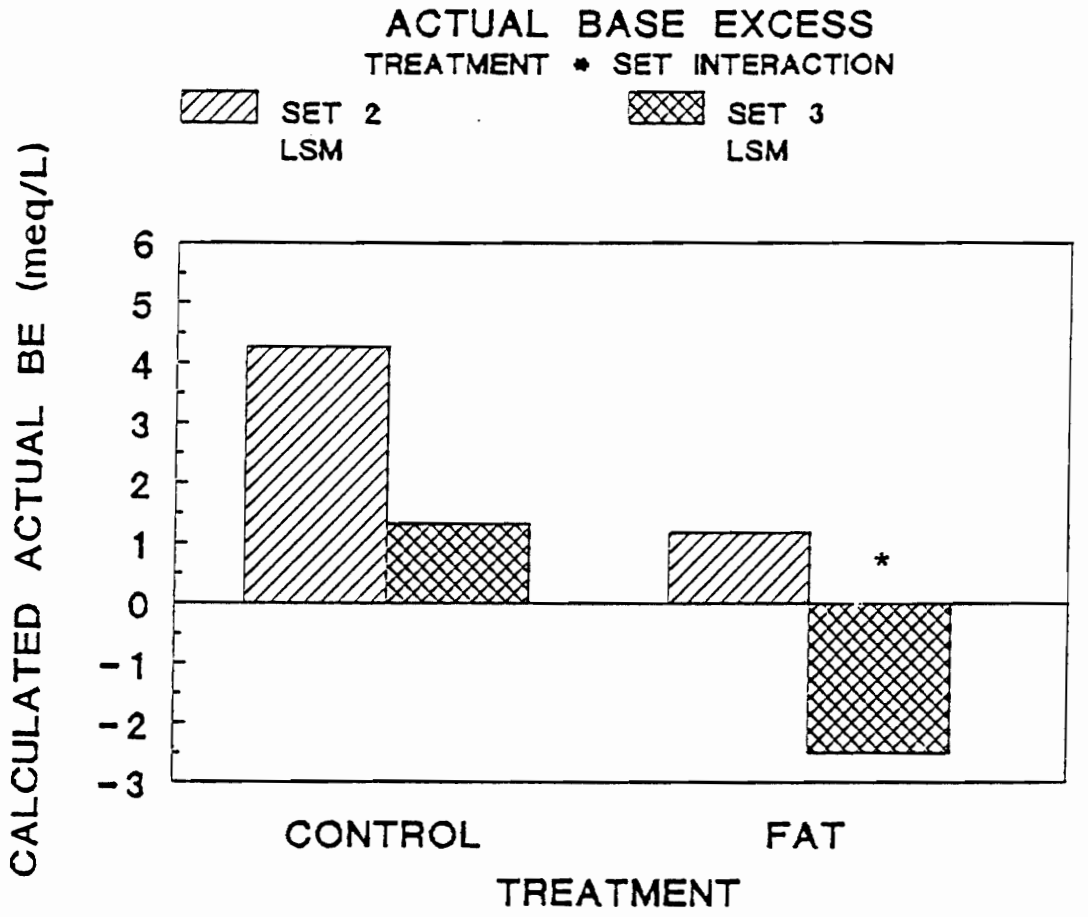

Figure 16. Treatment * SET interaction for base excess (SE: ± 1.47) * denotes significant difference ($p<.05$).

PLASMA STRONG IONS AND THE STRONG ION DIFFERENCE:

Results for the responses of the SID to exercise are shown in Figure 17. There was a small decrease in the overall SID from rest to exhaustion due to a large, significant ($p<.001$) 75% increase in lactate concentration, but there were no significant diet differences. There was also a small, significant ($p<.001$) rise in plasma potassium, an insignificant rise in plasma sodium, and a significant decrease ($p<.001$) in plasma chloride, which is shown in Figure 18. These three changes would all increase the SID, but could not overcome the overwhelming effects of the increased lactate, and therefore SID decreased. It is interesting to note that if lactate had not been measured, SID would have seemed to increase with exercise, as shown by Figure 19. This illustrates the importance of considering lactate when studying acid-base balance during exercise, and demonstrates why we cannot calculate the SID as only $[Na^+] - [Cl^-]$. Lactate changed very little at rest when comparing groups, but changed significantly in both groups of horses during exercise at both SETs 2 and 3 (Figure 20), with increases ranging from 63 - 80%.

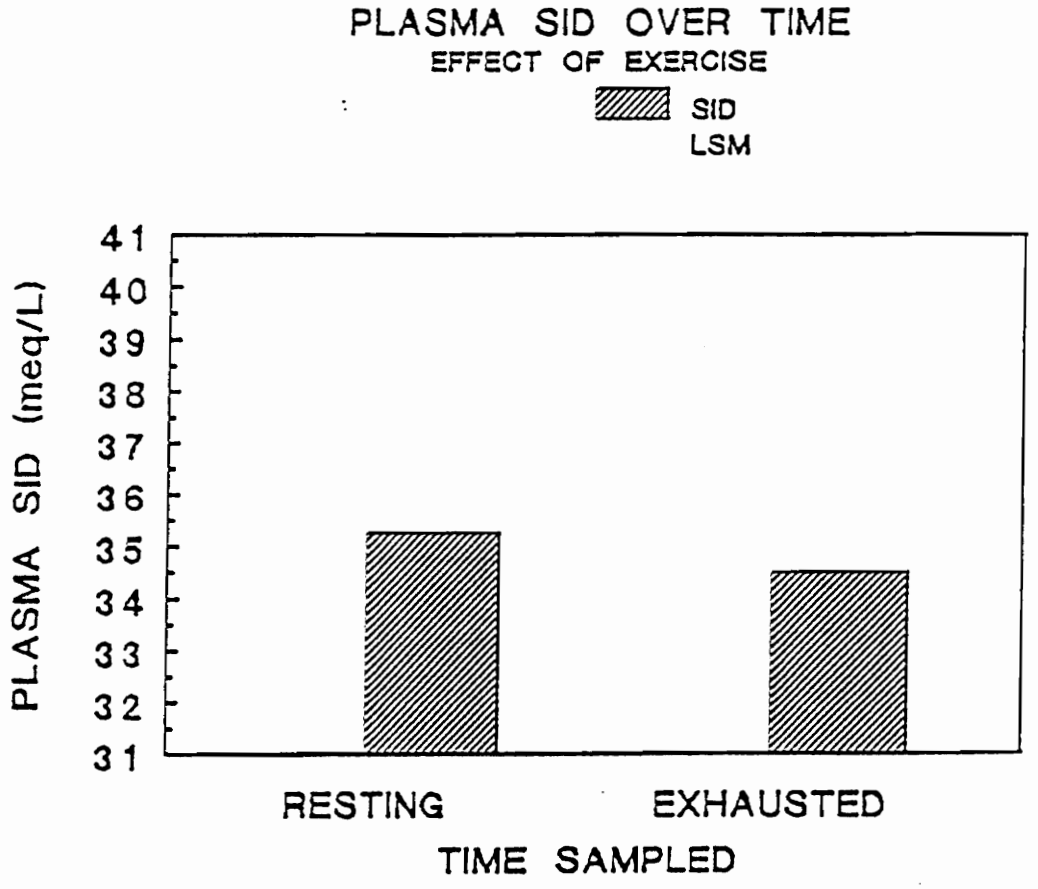

Figure 17. Effects of exercise on overall SID (SE: ± 2.33).

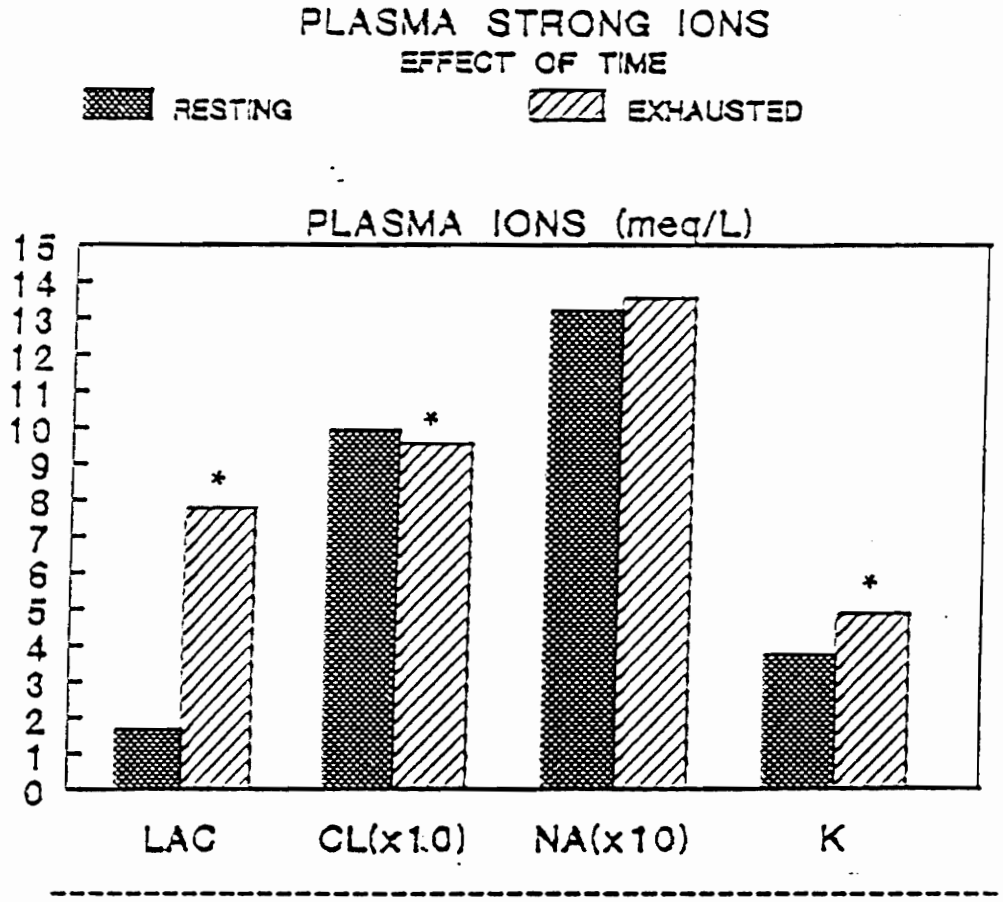

Figure 18. Effects of exercise on plasma strong ions (SE: $[Cl^-]$: ± 1.43; [Lactate]: ± 0.66; $[Na^+]$: ± 1.81; $[K_+]$: ± 0.25) * denotes significant difference from resting ($p<.05$).

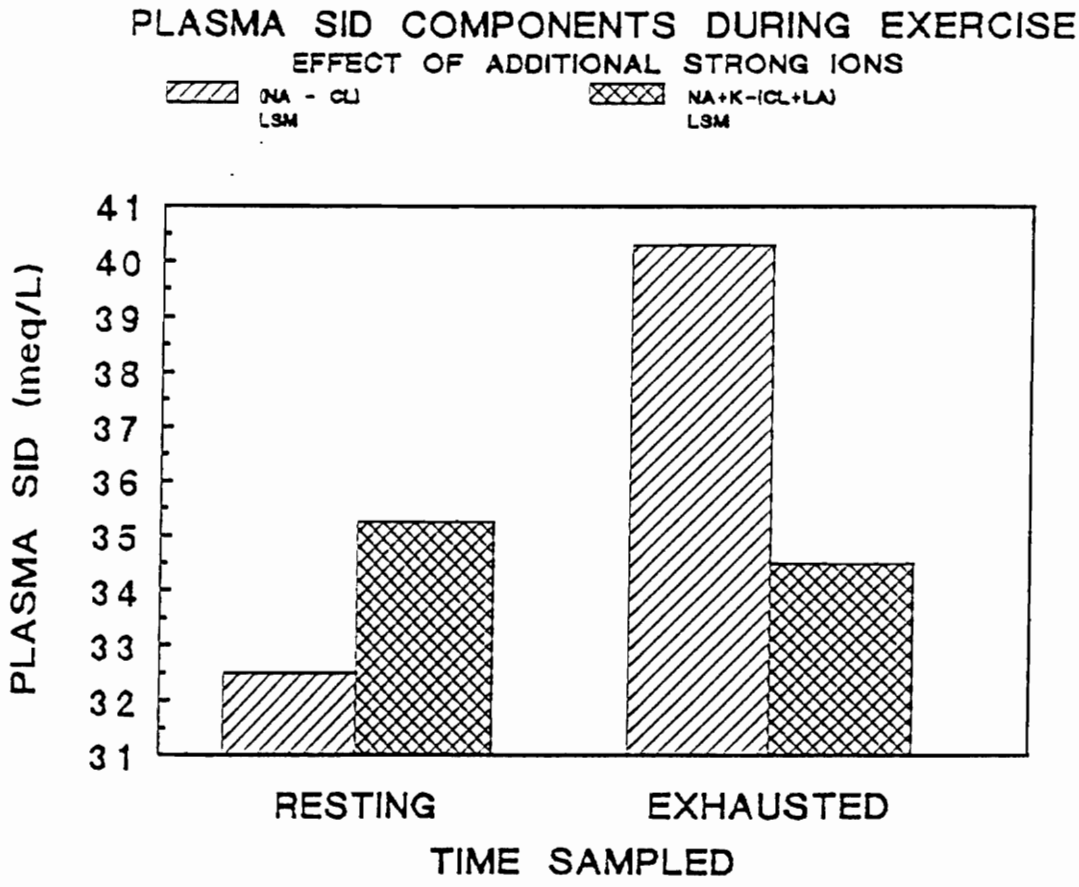

Figure 19. Evaluation of SID: Effects of exercise and evaluation with additional strong ions.

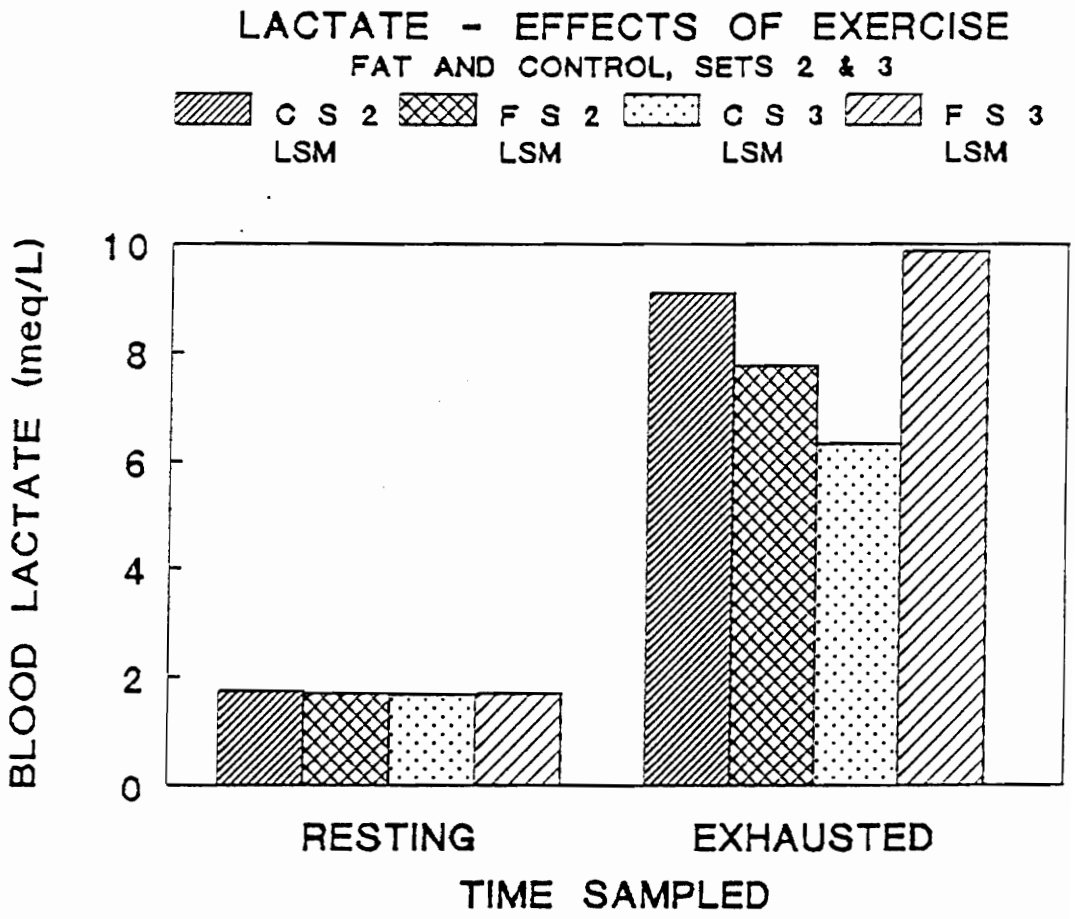

Figure 20. Effects of exercise and 15 weeks of training on blood lactate: control diet vs. fat diet (C S 2 = control group, SET 2; F S 2 = fat group SET 2; C S 3 = control group SET 3; F S 3 = fat group SET 3).

There was also a significant diet * SET interaction ($p<.05$), which indicated that the SID for the fat group experienced a slightly greater decrease than the SID for the control group between SETs 2 and 3 (Figure 21). This difference is probably due to the larger increase in blood lactate in the fat horses at SET 3.

These results give us a fairly detailed interpretation of what is happening in the exercising horse as compared to only looking at the classical measurements of pH, PCO_2, and bicarbonate. (A summary of all means at rest and exercise can be seen in Appendix Table 3). Early exercise is not very strenuous, and thus none of the variables measured over time change to any great extent, as can be seen by the graphs. The body can still adequately modify respiratory and metabolic changes through it's buffering systems at this point. The PCO_2 starts to take a large drop between 5 and 6 m/sec, possibly due to increased hyperventilation. Although the cause of this hyperventilation is unclear in the horse, it causes changes in the pH, bicarbonate, and base excess levels. Hypocapnia lowers the carbonic acid concentration of the body fluids, which should result in a fall in $[H^+]$, or an increase in pH (Gennari et al, 1982). Because the hypocapnia can be maintained by increased breathing frequency in the horse, it results in

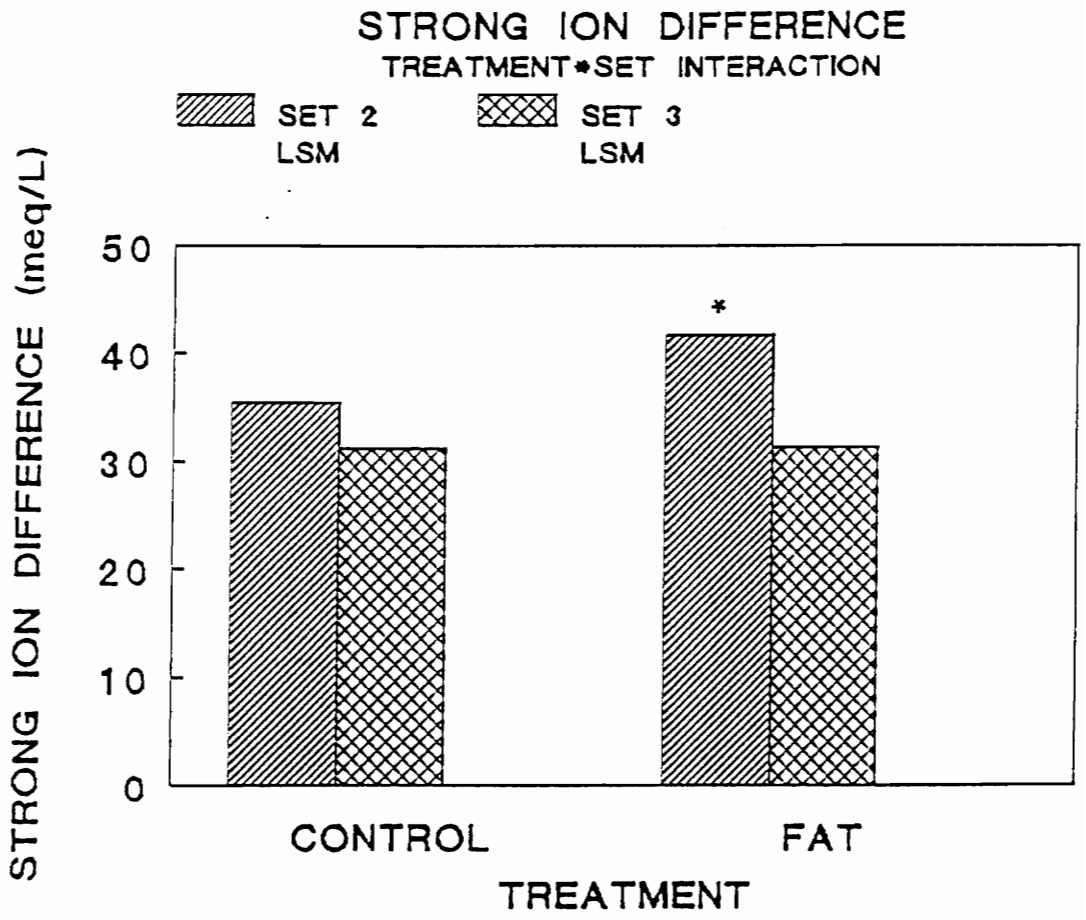

Figure 21. Treatment * SET interaction for plasma SID (SE: ± 1.96) * denotes significant difference ($p<.05$).

an open-ended system. This actually causes a decrease in the bicarbonate levels and the pH, as the bicarbonate is utilized to buffer acids from the working muscle. The products of this reaction (water and CO_2) are more easily handled by the body.

These responses have also been seen in the arterial blood of the horse. Pan et. al. (1986) noted that a fall in bicarbonate reflected the sum effect of a decrease in pCO_2, an increase in lactic acid, and the mobilization of splenic erythrocytes. Subsequent work by this group found that the equine is distinctly different from humans because the acute in vivo $[H^+]$ buffer capacity exceeds the in vitro buffer capacity (Forster et. al., 1990).

Although we were not able to obtain arterial blood from all the horses at all the SETs during this experiment, we did obtain arterial blood from one horse consistently throughout this trial. The right carotid artery had been previously relocated to a position near the skin, and was also catheterized in this animal. Samples were obtained in the same manner as the ones for the jugular vein, and samples were drawn simultaneously from the vessels during exercise. Responses of pH, pCO_2, and bicarbonate in the artery and vein of this horse during exercise and to training are shown in

Figures 22 -24, and sample comparisons of each variable in the two vessels are shown in Figures 25 - 27.

The arterial pH seemed slightly higher in this animal than the venous pH at both SETs. This may be because the artery is more representative of the muscle effluent, and shows a greater degree of acidosis. The arterial pCO_2 does not seem much different from the venous side until later in exercise, where it seems to be lower in both SETs. This may illustrate the effects of additional CO_2 from the head region, which would be found in the jugular vein. There do not appear to be any differences between vessels in the bicarbonate levels. The venous and arterial data from this horse were plotted and fit with linear regressions. The R values obtained were fairly high, with the best correlation being between venous and arterial bicarbonate (Figure 27). Other researchers have suggested that jugular venous sampling may be indicative of the whole body value for lactate in exercising horses (Miller et al., 1987), but differences between sites for blood gas values have been found in exercising horses (Parks and Manohar, 1984) and dogs with induced acidosis or alkalosis (Ilkiw et al., 1991). The results of this study may suggest that venous and arterial bicarbonate ($R = 0.93$) and venous and arterial

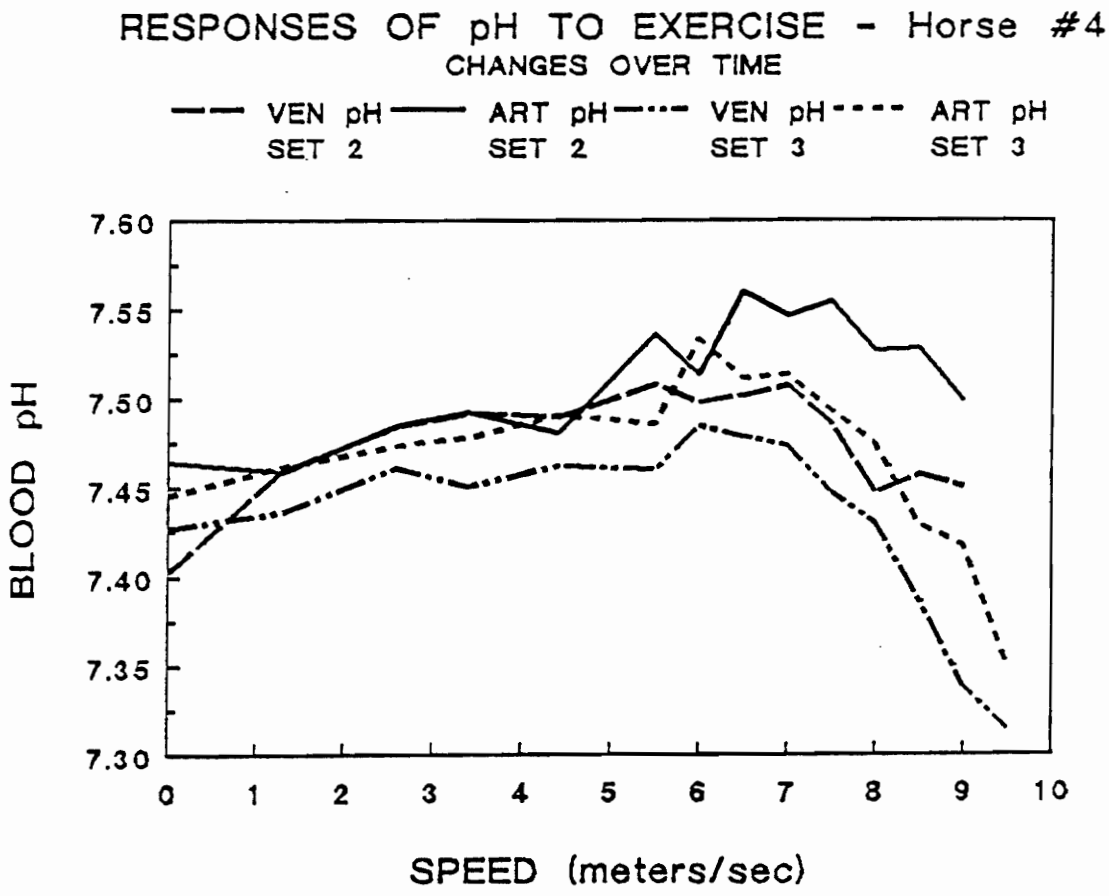

Figure 22. Responses of arterial and venous pH to 15 weeks of training and exercise in horse #4.

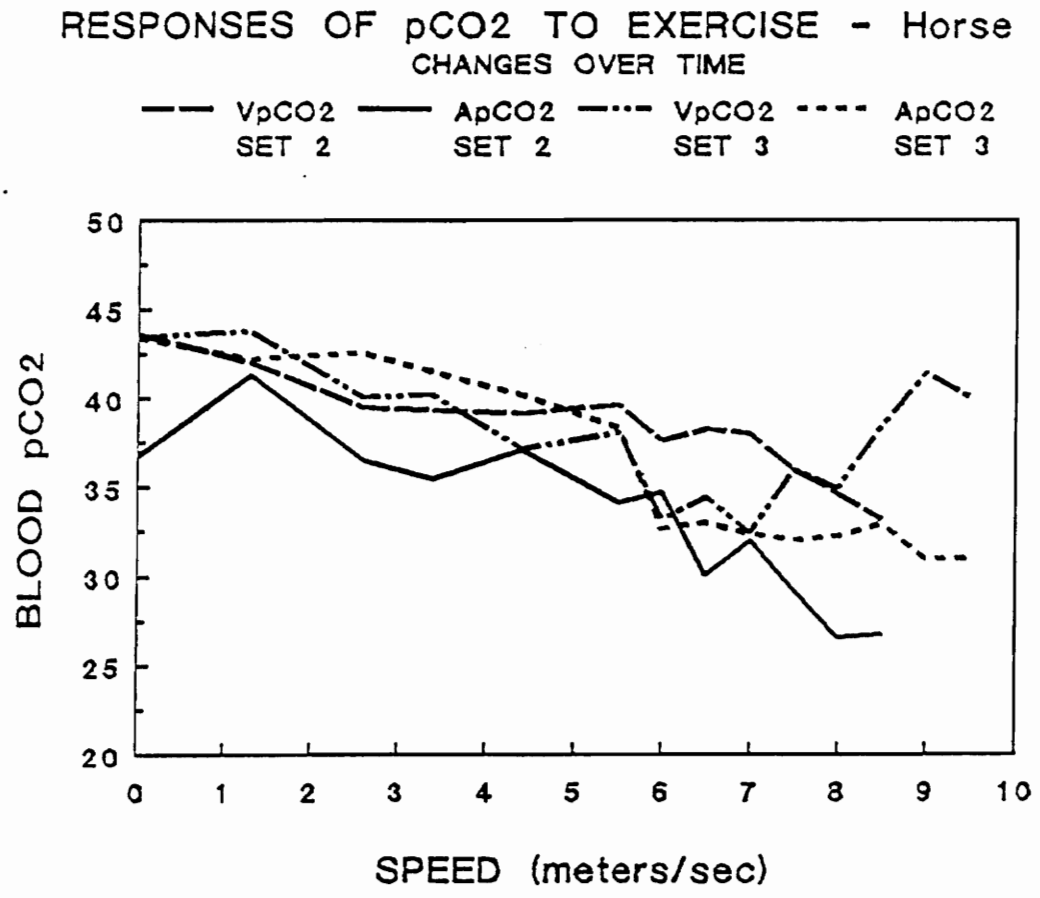

Figure 23. Responses of arterial and venous pCO_2 to 15 weeks of training and exercise in Horse #4.

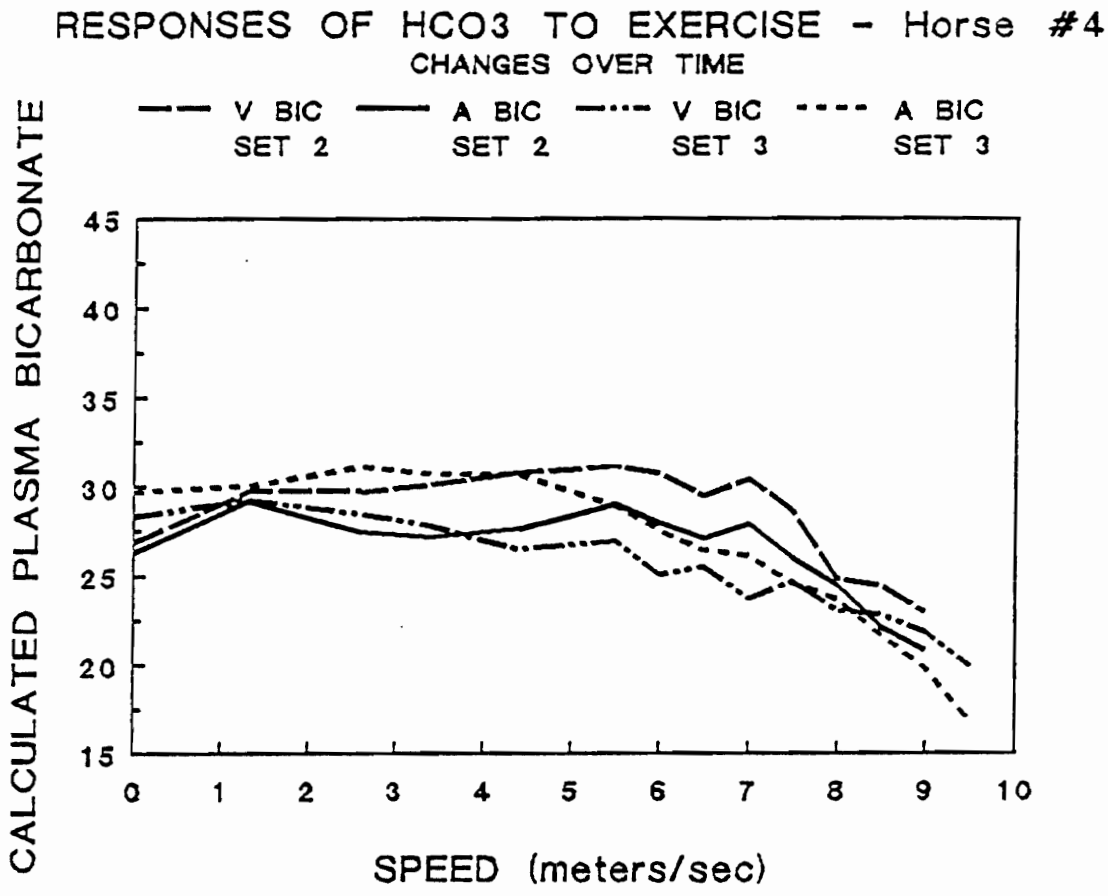

Figure 24. Responses of arterial and venous bicarbonate concentrations to 15 weeks of training and exercise in Horse #4.

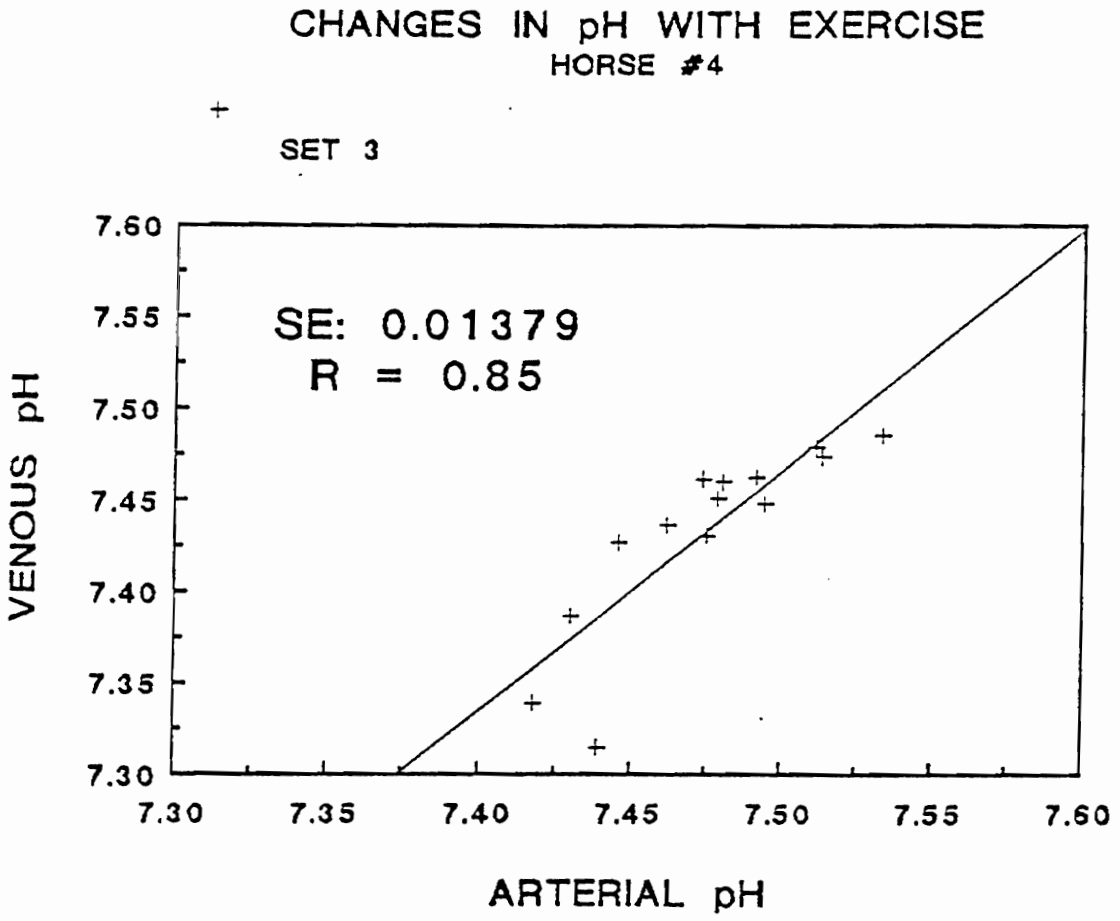

Figure 25. Relationship between arterial and venous pH in Horse #4 at SET 3.

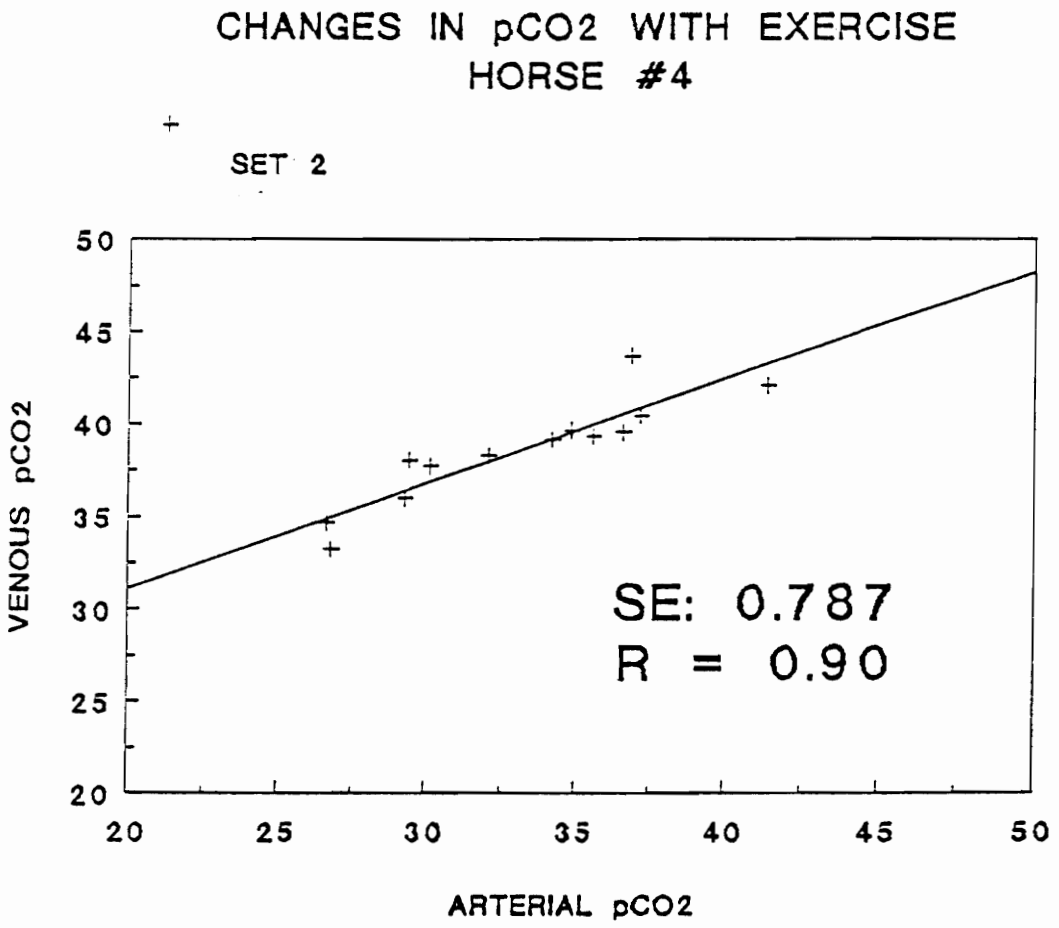

Figure 26. Relationship between arterial and venous pCO_2 in Horse #4 at SET 2.

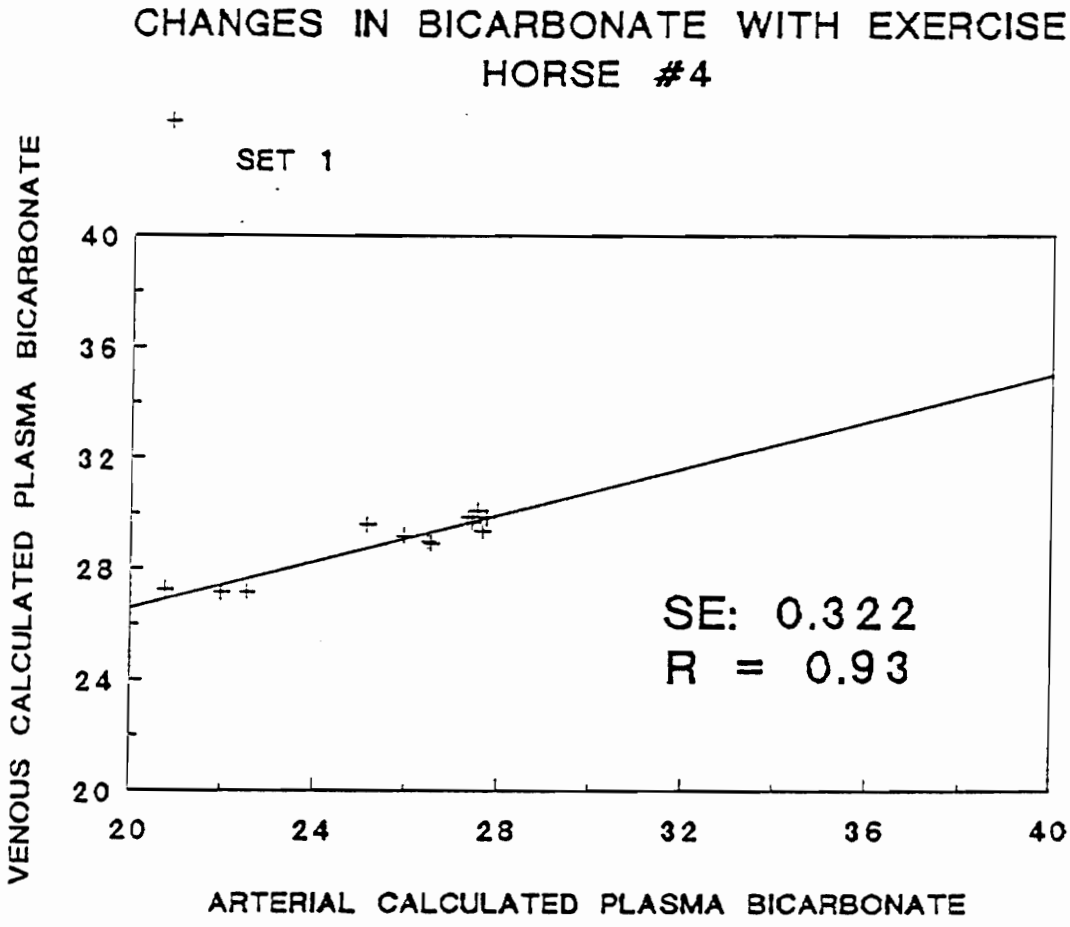

Figure 27. Relationship between arterial and venous bicarbonate concentrations in Horse #4 at SET 3.

pCO_2 are highly correlated (R = 0.90), but these results were only from one animal.

In agreement with our findings are the results of Snow and Mackenzie (1977) who found a decrease in venous pCO_2, pH, and bicarbonate, and a significant increase in total plasma protein, due in part to a decrease in plasma water volume. These results have also been found in humans undergoing maximal exercise (Hermansen et. al., 1984).

In contrast to our findings, another study by Forster et. al. (1990b) showed an arterial hypocapnia coupled with a decrease in arterial plasma $[H^+]$, and an increase in SID. These differences may be due in part to the lower exercise intensity employed in their project.

Because it is important to consider the many variables of fatigue during exercise, we also monitored rectal temperatures. The 7% average rise in mean rectal temperature during exercise is shown in Figure 28. It has been suggested that a high muscle temperature can contribute to muscle fatigue during exercise, and this value in horses is thought to be approximately 107 degrees F (Hodgson et al., 1990). The highest rectal temperature recorded in our horses was 106.5 degrees F, and is presumably lower than the muscle temperature. The increase in temperature may have contributed to fatigue.

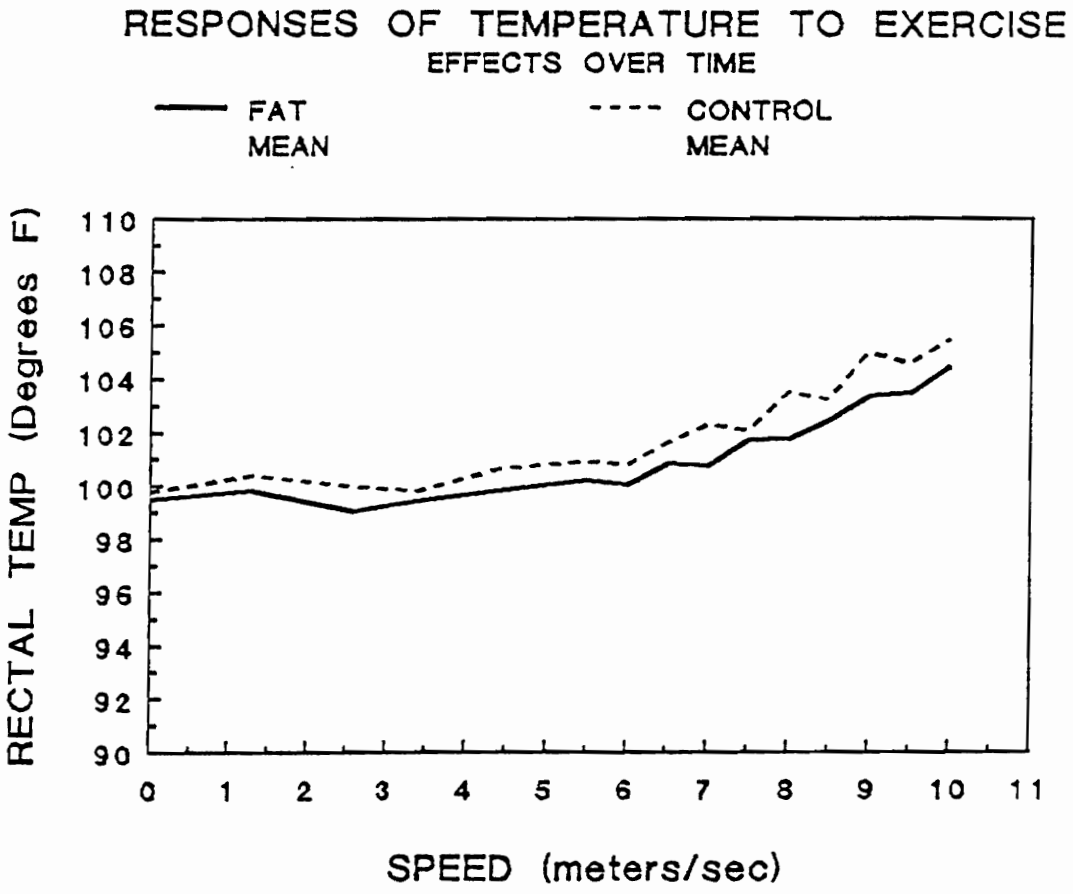

Figure 28. Responses of mean rectal temperature to exercise.

Changes observed in the strong ions and the SID during exercise in our study revealed more information about the movement of ions and their role in buffering fatigue. The increase in plasma $[K^+]$, most likely due to a loss from the muscle during exercise has been seen in dogs (Lade and Brown, 1963), cattle (Fosha-Dolezal and Fedde, 1988), humans (Sjogaard et al., 1985; Hespel et. al., 1986), and horses (Harris and Snow, 1988). Some researchers have suggested that a loss in $[K^+]$ from the cell may be a major factor in causing fatigue, due to a change in the membrane potential (Lindinger and Heigenhauser, 1991). Potassium may be moving out of the cell in exchange for H^+ ions, because if the extracellular pCO_2 is decreased, there will be a greater increase in H^+ ions outside the muscle cell, and K^+ ions will move out in exchange (Brown et al., 1963). This seems to be the response observed in our animals. However, it should be noted that the values obtained in our project do not take into account the possible fluxes in water between cells and plasma, and this factor could change some ion concentrations. For example, dehydration alone inside muscle cells can decrease the $[K^+]$ (Sjoraard et al., 1985).

The level of plasma chloride decreased with time, and this may be due in part to losses through sweat. It

has also been shown that chloride may be able to move freely across the cell membrane, and could have been re-distributed (McCaig and Leader, 1984). The small, insignificant rise in plasma sodium has also been seen in humans with exercise (Hespel et al., 1986), but the cause of this rise is unknown. It may also be due to shifts in other ion concentrations, and the subsequent water shifts in the body.

The rise in lactate with exercise was expected, but the mechanism of lactate transport out of the muscle is still unclear. Anion exchange may result in an efflux of lactate out of the muscle, accompanied by uptake of chloride or bicarbonate ions (Jackson, 1990). There is some evidence that suggests lactic acid may be removed from the cell as a molecule by a carrier after exercise has ceased (Juel, 1988). If lactate is being exchanged for chloride or bicarbonate, this may be a contributing factor to the decreases found in these metabolites.

The overall goal of the many fluxes and exchanges seen in the body is to help control the intracellular $[H^+]$. The acids must be brought out of the working cell and subsequently neutralized. The total acid load in the blood has a respiratory component (CO_2), and a metabolic component (strong ions). If we evaluate the changes seen in the horses during exercise according to the SID

approach, we would look at the following independent variables: 1) The small decrease in plasma SID with exercise (metabolic acidosis); 2) The decrease in pCO_2 with exercise (respiratory alkalosis); and 3) The small rise in plasma albumin (metabolic acidosis). These changes, according to Stewart (1983), should cause a decrease in the pH and the bicarbonate, which is what was found in this study. It is also important to note that when the SID approach is used, it is possible to now demonstrate a respiratory alkalosis combined with a truly metabolic acidosis, as shown by the pCO_2 and SID, respectively. If evaluation of acid-base status involves bicarbonate concentration as the sole metabolic component, we can see that this may lead to large errors in interpretation. Bicarbonate concetration depends upon changes in the pH and the pCO_2, and cannot be considered an exclusively metabolic component. The SID observations should be of value during exercise in many species, and it may be possible to predict certain metabolic changes. Therefore, the overall evaluation of acid-base balance during exercise should include a measurement of the SID.

The lack of significant diet effects on the variables studied was disappointing, but may have been due in part to the low number of experimental animals. There were some interesting diet and training

interactions, and the results obtained emphasize the importance of the early development of hypocapnia in the horse. This strong reflex may be able to mask any diet effects when looking at pCO_2 in the venous blood. The trend for increased blood lactate in the fat group with training seems promising, and may help confer some advantages to the animal during exercise. Finally, this study illustrates the importance of using the SID approach when evaluating acid-base balance during exercise. Although the variables studied here did not give a good indication of enhanced fat oxidation in the exercising horse supplemented with a high level of dietary fat, some of the trends and interaction effects suggest that there are changes going on in the animal, and emphasizes the need for further research in this area.

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APPENDIX TABLE 1. TRACE MINERALIZED SALT (EQUI-CHOICE) GUARENTEED ANALYSIS

	(%)
Calcium (Max)	9.000
Calcium (Min)	7.000
Phophorus	8.000
Magnesium	1.000
Sulfur	1.000
Zinc	0.500
Iron	0.300
Copper	0.120
Manganese	0.100
Cobalt	0.002
Iodine	0.002
Salt (Max)	36.00
Salt (Min)	34.00
Sodium	13.65
Chlorine	21.35
Selenium	0.001
Vitamin A	330,000 IU/kg
Vitamin D	56,100 IU/kg
Vitamin E	660 IU/kg

APPENDIX TABLE 2. BODY CONDITION SCORES[a]

Score	Condition	Appearance
1	Poor	Extremely emaciated Projecting ribs and tailhead No fatty tissue can be felt
2	Very thin	Somewhat emaciated Slight fat over spine Prominent ribs and tailhead
3	Thin	Some fat buildup on spine Slight fat over ribs Cannot identify vertebrae
4	Moderately thin	Slight ridge over back Faint outline of ribs
5	Moderate	Back is flat Ribs not easily visible Withers rounded
6	Moderately fleshy	Spongy fat over ribs Fat deposits along back Fatty tissue around withers
7	Fleshy	May have crease down back Spongy fat over tailhead Noticeable fat between ribs
8	Fat	Crease down back Difficult to feel ribs Very soft fat over tailhead
9	Extremely fat	Patchy fat over ribs Bulging fat around withers Bulging fat around neck

[a] Adapted from Henneke et. al. (1983)

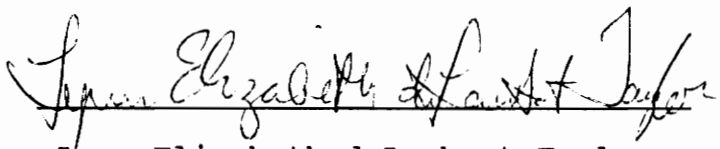
APPENDIX TABLE 3. EFFECTS OF EXERCISE ON BLOOD METABOLIITES (MEASURED AT REST (T_0) AND EXHAUSTION (T_E))

	Control[b]				Fat[c]			
	Set #2		Set #3		Set #2		Set #3	
Metabolite[d]	T_0	T_E	T_0	T_E	T_0	T_E	T_0	T_E
pH	7.44	7.39	7.43	7.38	7.41	7.38	7.41	7.33
PCO_2 (mmHg)	43.9	37.0	42.7	38.1	45.6	41.6	44.7	44.8
HCO_3^-(meq/L)	30.3	22.7	28.5	19.8	27.8	23.8	27.3	22.1
ALB (g/dl)	3.52	3.78	3.54	3.89	3.36	3.52	3.68	3.69
ABE (meq/L)	4.89	0.04	3.65	-3.05	2.57	-3.72	2.06	-4.13
HR (BPM)	40	191	38	192	43	193	39	211
HB (g/dl)	11.9	15.6	12.8	14.0	10.2	15.0	13.3	15.4
Strong ion difference components[e]:								
Metabolite[f]								
Sodium	134	139	135	138	131	132	127	133
Potassium	3.69	4.97	4.07	5.09	3.57	4.39	3.63	4.89
Chloride	103	101	97	93	98	94	100	95
Lactate	1.73	9.1	1.69	7.76	1.68	6.33	1.69	9.86
SID	36.7	34.2	40.9	42.5	34.9	27.4	28.5	33.9

[a] Least squares means
[b] Mean of 4 animals
[c] Mean of 3 animals
[d] pH, PCO_2, HCO_3^-, ABE (Actual base excess), HB (Hemoglobin), in venous blood; ALB (Albumin) in venous plasma; HR (Heartrate);
[e] All are meq/L
[f] Na, K, Cl, in venous plasma; Lactate in venous blood;

VITA

Lynn Elizabeth deLambert Taylor, daughter of Elizabeth and Don deLambert was born on January 5, 1966 in Eglin, Florida. Upon graduation from Brookfield High School, Brookfield, Connecticut, the author entered the University of Connecticut in September, 1984. The author received her Bachelor of Science in Animal Science in December, 1988. The author continued to pursue her education as a candidate for a Master of Science degree in Animal Science (Equine Nutrition and Exercise Physiology) at Virginia Polytechnic Institute and State University in Septenber, 1989. The author married Michael Anthony Taylor of Cheshire, Connecticut on August 16, 1991.



Lynn Elizabeth deLambert Taylor

THE EFFECTS OF ADDED DIETARY FAT ON ACID-BASE STATUS IN EXERCISING HORSES

by

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(ABSTRACT)

Two groups of horses were each fed either a control diet of ground hay and concentrates (4 horses), or a similar diet with 10% added fat after undergoing a baseline Standard Exercise Test (SET). The SET was a stepwise, incremental test to exhaustion on an equine treadmill set at a 6% slope. Resting and working heartrates and rectal temperatures were monitored, and venous blood was collected at rest, and every 3 minutes during exercise, just prior to each speed change. Blood was analyzed for pH, hemoglobin, and pCO_2, and base excess and plasma bicarbonate levels were calculated using nomogram equations. Plasma samples were analyzed for albumin at each step, and for sodium, potassium, chloride, and lactate at rest and exhaustion only. The plasma SID was calculated at rest and exhaustion by the following equation:

$$([Na^+] + [K^+]) - ([Cl^-] + [Lactate])$$

The SET was performed after 16 days of interval training, and once more after another 16 days of interval training. Differences over time during exercise were found: heartrate, lactate, and potassium increased ($p<.001$), and hemoglobin increased ($P<.01$). Decreases were found in pH, pCO_2, bicarbonate concentration, base excess, and chloride ($p<.001$). Training effects were found in resting and working heartrates, pCO_2, bicarbonate concentration, and base excess, which all decreased during exercise with training. Hemoglobin increased during exercise with training. There were treatment * SET interactions for Strong Ion Difference, base excess, lacate concentration, pCO_2, and pH. There were no differences found between groups for any of the variables measured. Both groups showed improvements in fitness with training, and the fat group had a higher level of plasma lactate by SET 3. These results suggest that a high fat diet combined with interval training may have some effects on plasma lactate, and that training alone can affect many variables. The results also give evidence to support the evaluation of SID during exercise in horses.