

NITROGEN UTILIZATION AND METABOLIC RESPONSES OF PONIES
TO INTENSE ANAEROBIC EXERCISE

by

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Chapter 1

INTRODUCTION

Currently an increasing number of horses are produced for use in racing and other performance events. Certainly those persons involved in these aspects of the horse industry are interested in maximizing the horse's performance potential. This is not possible, however, without a thorough understanding of the relationship of nutrition to exercise. A substantial amount of research has been done in the area of equine nutrition, and to a more limited extent in equine exercise physiology. Nutrition and exercise are very much related, however, and it has been shown that as the intensity and duration of work increase, the energy requirements of the horse increase as well. While this point is virtually undisputed, a more controversial point is whether or not the horse's protein requirements increase as a result of increasing workload. Based primarily on research done in draft horses (Harvey et al., 1939) and endurance trained horses (Slade et al., 1975), the National Research Council (NRC, 1978) has recommended that the exercising horse does not require additional dietary protein above the maintenance level of 8.5% crude protein/d. However, recent studies done by

Citations in this thesis follow the style of the Journal of Animal Science.

Freeman et al. (1985a) indicate that nitrogen retention tends to increase during strenuous exercise. If this is the case, increasing protein intake in horses doing strenuous work might enhance athletic performance.

Research in human athletics has shown that muscle mass and strength increase as a result of anaerobic weight training due to increased protein synthesis and muscle fiber hypertrophy (Gollnick et al., 1973; Consolazio et al., 1975; Brooks and Fahey, 1984). If this is true in the horse, increased protein intake might be utilized as muscle building material, and an increase in nitrogen retention, as well as possible changes in muscle fiber size or type, could result from performing anaerobic-type work.

Since a great number of horses are used in anaerobic-type events, such as cutting, roping, and quarter-mile racing, it is important to know not only if nutrient requirements are being met, but also what the best training strategies are for maximizing performance. Clearly, it is necessary to investigate protein utilization during exercise, as well as different training regimens, to assist the horse in reaching its ultimate performance potential.

The objectives of this study were to investigate the effect of intense anaerobic exercise on nitrogen utilization in ponies, and to determine cardiovascular and muscular adaptations to this exercise.

Chapter 2

REVIEW OF LITERATURE

Heart Rate in Response to Exercise

Heart rate is commonly used to assess physical fitness in man (Cotton, 1932; Karpovich, 1965; Gontzea et al., 1973; Astrand and Rodahl, 1977; Brooks and Fahey, 1984) and in the horse (Asheim et al., 1981; Sigler, 1981; Rodiek et al., 1982; Erickson et al., 1985). Trained human athletes have lower resting heart rates than untrained individuals (Cotton, 1932; Karpovich, 1965; Brooks and Fahey, 1984). In the horse, however, resting heart rate does not seem to be affected by training (Cardinet et al., 1963; Pearson, 1980; Sigler, 1981; Erickson et al., 1985; Sexton et al., 1985), although Stewart (1972) observed a nonsignificant trend toward lower resting heart rates after a training regimen in Thoroughbred race horses, and Marshland (1968) observed reduced resting heart rates in Standardbred trotters after endurance training. Similarly, Burke et al. (1981) found a nonsignificant trend for reduced heart rates in 2 and 3 yr old Quarter Horse fillies following a 30 d training program.

Heart rate responses to different types of exercise may be of some use in evaluating fitness. As Brooks and Fahey (1984) point out, the rate and extent of the heart rate elevation depend on several factors including age, sex,

fitness level and type of exercise. In the horse, increased heart rate during different types of work has been observed (Asheim et al., 1970; Lindholm and Saltin, 1974; Pearson, 1980; Sigler, 1981; Thomas and Fregin, 1981; Miller et al., 1985; Topliff et al., 1985) and is affected by both intensity (Pearson, 1980) and duration (Ehrlein et al., 1973; Lindholm and Saltin, 1974) of exercise. Submaximal exercise training results in lowered heart rates during exercise in both horses and humans (Asheim et al., 1970; Ehrlein et al., 1973; Sigler, 1981; Brooks and Fahey, 1984; Erickson et al., 1985; Sexton et al., 1985).

In the horse, maximum heart rate may exceed 240 beats/min (Lindholm and Saltin, 1974) which is high in comparison to other species (Asheim et al., 1970; Ehrlein et al., 1973; Brooks and Fahey, 1984). Brooks and Fahey (1984) point out that maximum heart rate in humans is relatively stable. It is only slightly affected by training (a decrease of about 3 beats/min) and tends to decrease with age. Asheim et al. (1970) found that the maximum heart rate of horses was highly variable, and did not seem to be related to size or age of the horse. Thus, due to this variability, data interpretation may be difficult when using a submaximal exercise test to compare horses.

During submaximal exercise, a distinct pattern of heart rate response is observed (Marshland, 1968; Lindholm and Saltin, 1974; Pearson, 1980; Sigler, 1981; Thomas and

Fregin, 1981). There is an immediate rise in heart rate followed by a plateau as long as the work load is held constant. A reduction in the height of this plateau was produced by conditioning in man (Clausen et al., 1970) and in the horse (Marshland, 1968; Asheim et al., 1970; Sigler, 1981).

The effect of training on recovery heart rate after exercise is somewhat questionable. Cardinet et al. (1963) and Stewart (1972) suggested that recovery of heart rate after exercise appeared to take longer in unfit versus fit horses. Sigler (1981) found that after 28 d of galloping (3.62 km in 10 min), horses had a significant decrease in 5 min and 10 min recovery heart rates following a 30 min exercise tolerance test on the treadmill. Similarly, Sexton et al. (1985) found a significant decrease in 1, 6, 11 and 16 min recovery heart rates after a 25 min treadmill test in ponies that had undergone 8 wk of endurance and interval training. However, Milne et al. (1977) found no significant differences in mean recovery heart rate values in Standardbred trotters from the beginning to the end of a 92 d training program. In addition, Brooks and Fahey (1984) indicate that post-exercise heart rates can be a rough measure of fitness, and although fit individuals tend to recover more quickly, the rate of recovery is dependent on maximum heart rate, duration of exercise and intensity of exercise. Banister and Purvis (1968) found that recovery

heart rates were not indicative of fitness in horses doing less strenuous types of work. Furthermore, Witherington (1971) found a tendency for track and climatic conditions during exercise to affect recovery heart rates in Thoroughbred race and steeplechase horses.

Respiration Rate in Response to Exercise

Respiration rate is not a reliable indicator for evaluating fitness due to the many factors that affect respiration, including climate, lactic acid concentration in the blood and depth of breathing (Cardinet et al., 1963; Brooks and Fahey, 1984). Respiration rate does increase during exercise in the horse (Milne et al., 1976; Snow and MacKenzie, 1977a; Pearson, 1980; Sigler, 1981; Campbell et al., 1985; Topliff et al., 1985). Cardinet et al. (1963) found much variability in rate and depth of respiration in endurance horses after a ride, but noted that breathing was more shallow as respiration rate increased. However, it was also noted that there were greater differences in respiration rates than in heart rates when comparing horses that were able to complete the ride. In addition, Hinton (1978) found that the best indication of exhaustion in trail horses was the 30 min recovery respiration rate. However, Kelly (1977) found that heat stress resulted in much variability in respiration, and thus, heart rate during recovery was a more accurate measure of exhaustion than

recovery respiration rate in endurance horses. Similarly, Stewart (1972) noted irregular respiration rates at 10 to 20 min post-exercise in race horses. Snow and Mackenzie (1977a), Pearson (1980) and Sigler (1981) also observed large variations in respiration during exercise and recovery and found no significant changes in respiration rate in horses due to conditioning.

Blood Lactic Acid in Response to Exercise

Blood lactic acid concentration has often been used as an indicator of fitness in humans (Wasserman et al., 1967; Karlsson and Saltin, 1970; Donovan and Brooks, 1983) and in horses (Asheim et al., 1970; Lindholm and Saltin, 1974; Snow and Mackenzie, 1977b; Keenan, 1979; Pearson, 1980; Sigler, 1981; Thomas and Fregin, 1981; Erickson et al., 1985; Sexton et al., 1985; Topliff et al., 1985). Accumulation of lactic acid seems to be related to both the intensity and the duration of exercise (Astrand and Rodahl, 1977; Brooks and Fahey, 1984). The energy needed for high intensity, short duration exercise is supplied primarily by anaerobic metabolism, while the energy required for lower intensity long duration exercise is supplied mainly by aerobic metabolism (Wasserman et al., 1967; Astrand and Rodahl, 1977; Brooks and Fahey, 1984). During the first few minutes of low or moderate intensity aerobic exercise, blood lactate levels increase and then reach a plateau which remains

fairly constant for the duration of the exercise (Knuttgen, 1962). Anaerobic metabolism supplies energy at the beginning of the exercise, then aerobic metabolism takes over and completely meets the energy needs (Astrand and Rodahl, 1977). Lactic acid, then, is produced as an end product of anaerobic energy metabolism in the muscle, and will continue to build up in the muscle and blood as exercise intensity increases (Brooks and Fahey, 1984). This rise in blood lactate level with increasing exercise intensity has been observed in humans (Wasserman, 1967; Karlsson and Saltin, 1970; Brooks and Fahey, 1984) and in horses (Persson, 1967; Asheim et al., 1970; Lindholm and Saltin, 1974; Pearson, 1980; Sigler, 1981, Sexton et al., 1985).

In human athletes, as the intensity of the exercise increases, oxygen consumption increases linearly, but blood lactate level is not altered until 60 to 70% of VO_2 max has been reached (Brooks and Fahey, 1984). Thus, the increase in blood lactate level is nonlinear. In the past, the inflection point on the blood lactate curve has been called the anaerobic threshold, but Brooks and Fahey (1984) point out that the inflection point does not indicate the onset of anaerobic metabolism, it simply reflects the balance between entry and removal of lactate from the blood. Hagberg et al. (1982) observed that McArdle's syndrome patients (persons incapable of forming lactic acid) had ventilatory inflection

points at a similar relative workload as normal subjects, even though there was no change in blood lactate level, thus indicating that the ventilatory or anaerobic threshold is not solely due to lactate accumulation. The factors responsible for the lactate inflection point during exercise of increasing intensity, then, are the recruitment of fast twitch glycolytic muscle fibers, hormonally mediated accelerations in glycolysis and glycogenolysis and a redistribution of blood flow from lactate-removing, gluconeogenic tissues to lactate-producing, glycolytic tissues (Brooks and Fahey, 1984). After exercise ceases, most of the blood lactate is oxidized, but some is incorporated into amino acids and proteins or is used as a gluconeogenic precursor (Brooks and Fahey, 1984).

In the exercising horse, lactate appears to accumulate in the blood at a heart rate of at least 150 to 158 beats/min (Persson, 1967; Moore et al., 1976). Most research indicates that blood lactate levels during or after exercise are decreased by conditioning. Milne et al. (1976) found that after a 6 wk training period, Standardbred horses had lower blood lactate concentrations following a submaximal trotting test. Pearson (1980) found a significant decrease in lactate production following a 30 min treadmill test in Quarter Horse mares that had undergone 28 d of gallop training, (3.62 km at 22 km/h). Sigler (1981) reported similar findings in Quarter Horse mares

after 28 d of galloping. Decreases in blood lactate response to treadmill tests following conditioning have also been reported in ponies (Sexton et al., 1985) and in 2 yr old Quarter Horses (Erickson et al., 1985). However, after a 10 wk training program, Snow and MacKenzie (1977b) saw no change in lactate response following a 22.4 km cantering session in Thoroughbreds and an 11.2 km cantering session in heavy hunters. Snow and MacKenzie (1977a) also found that after training, Thoroughbreds and heavy hunters had higher blood lactate concentrations in response to three 600 m maximal tests with 5 min rest periods between tests.

There is some question as to whether or not resting blood lactate levels change as a result of conditioning. Lindholm and Piehl (1974) suggest that in horses, a decrease in blood and muscle lactate may occur with training. However, Milne et al. (1976, 1977) found no significant change in resting lactate levels of Standardbred horses over a 42 and a 92 d training period, respectively. Pearson (1980) and Sigler (1981) observed no change in resting lactate values in Quarter Horse mares following 28 d training regimens. Erickson et al. (1985) and Sexton et al. (1985) reported similar findings. This agrees with work done in humans by Robinson and Harman (1941) who found that resting blood lactate levels were similar in trained and untrained individuals.

Muscle Physiology in Response to Exercise

Skeletal muscle constitutes about 35 to 65% of the carcass weight of meat-type animals (Forrest et al., 1975). By acting as a chemomechanical transducer, skeletal muscle converts chemical energy to mechanical and heat energy. However, this process is only about 30% efficient, so approximately 70% of the energy produced by the muscle is released as heat (Brooks and Fahey, 1984).

Skeletal muscle is composed of individual muscle fibers which are arranged in bundles and further subdivided into myofibrils and myofilaments (Briskey et al., 1970; Forrest et al., 1975; Brooks and Fahey, 1984). Contraction basically occurs when cross-bridges extend from myosin to actin and a conformational change occurs in the cross-bridge (Huxley, 1969).

During exercise many physical and chemical changes occur in the muscle, including glycogenolysis, lactate production and protein turnover (Laurent and Millward, 1980; Brooks and Fahey, 1984; Harris et al., 1984; Hodgson et al., 1985). Chemically, muscular activity leads to increased amounts of phosphocreatine, glycogen and myoglobin, while physically it leads to structural changes as well as increased contractile strength and endurance (Karpovich, 1965; Goldspink, 1970). The sarcolemma of the muscle fiber thickens and capillarization increases (Karpovich, 1965). In addition, muscle protein concentration is altered.

O'Shea (1976) found that low intensity, long duration exercise caused an increase in myofibrillar protein concentration. Furthermore, increases in both muscle size and mitochondrial size and mass are often seen as a result of muscular activity (Karpovich, 1965; Goldspink, 1970; Gollnick et al., 1973; Nimmo and Snow, 1982).

Forrest et al. (1975) point out that there does not appear to be any significant increase in the number of muscle fibers after birth, and thus, postnatal muscle growth is accomplished mainly by hypertrophy. Many other researchers share this belief (Rasch and Pierson, 1962; Goldspink, 1970; Laurent and Millward, 1980), however Edgerton (1978), Gonyea (1980) and Ho (1980) have reported fiber splitting, as well as hypertrophy, in response to high intensity resistance training. The increase in cell number due to fiber splitting is still highly controversial, however. Hyperplasia has been seen in rats and mice due to the DNA production and mitotic division of small mononucleated cells, called satellite cells (Mauro, 1961; Enesco and Puddy, 1964; Church, 1969). Still, in humans and horses, most muscular growth is due to hypertrophy, or muscle enlargement in response to overload stress. An increase in myofibrillar size and number results in a subsequent increase in muscle fiber size (Brooks and Fahey, 1984). Finally, muscle hypertrophy during strength training results in several biochemical changes, which include

increased levels of muscle glycogen, creatine, phosphorylase, ATP, ADP, creatine phosphate, phosphofructokinase and Krebs cycle enzyme activity (Brooks and Fahey, 1984).

Classification of Muscle Fiber Type and Changes Due to Exercise

Much research has been conducted to determine the muscle fiber types in skeletal muscle. Nystrom (1968) assessed succinic dehydrogenase, phosphorylase, glycogen, lipids, NADH₂-tetrazolium reductase and myofibrillar ATPase in cat muscle, and found three distinct fiber types. Guth and Samaha (1970) also identified three different muscle fiber types based on contraction speed. Finally, Ashmore and Doerr (1971) came up with a useful system for fiber typing based on findings by several researchers. The system included the ATPase staining technique (Guth and Samaha, 1970) and also the reduced diphosphopyridine nucleotide tetrazolium reductase (DPNH-TR) method for staining muscle fibers (Engel and Brooks, 1966). Farber et al. (1956) found that tetrazolium salts stain for oxidative enzymes as well as TPN and DPN diaphorase systems by forming diformazan granules. The nitroblue tetrazolium used in this method was also used by Novikoff et al. (1961) to describe the localization of oxidative enzymes in the mitochondria. Using the ATPase and DPNH-TR stains on muscle serial sections,

Ashmore and Doerr (1971) found three fiber types which they classified as alpha-red (fast twitch, high oxidative), beta-red (slow twitch, high oxidative), and alpha-white (fast twitch, low oxidative). The intermediate fibers (alpha-red) stained darkly for ATPase at pH 10 and had a high diformazan granule concentration. The white fibers (alpha-white) also stained darkly for ATPase, but had a low diformazan granule concentration. Finally, the red fibers (beta-red) stained lightly for ATPase and had a high diformazan granule concentration. There are many differences between the red and white fibers. White fibers contract more quickly than red fibers, are larger in size, have a higher glycogen content and exhibit more myofibrillar ATPase, phosphorylase and glycolytic enzyme activity. The red fibers contract more slowly, are smaller in size, have a higher concentration of mitochondria and exhibit more oxidative enzyme activity (Beatty and Bocek, 1970; Forrest et al., 1975; Brooks and Fahey, 1984). Recently, more descriptive names have been given to the different muscle fiber types: fast twitch glycolytic (FG), which are alpha-white, fast twitch oxidative glycolytic (FOG), which are alpha-red, and slow twitch oxidative (SO), which are the beta-red (Snow and Guy, 1980; Kline and Bechtel, 1983; Brooks and Fahey, 1984).

Research has shown that muscle fiber composition may be affected by exercise. Some researchers believe that endurance exercise results in an increased percentage of red

fibers and a decreased percentage of white fibers (Barnard et al., 1970; Ingjer, 1979). Prince et al. (1976) suggested that the opposite occurs with anaerobic training. Some researchers, however, observed no change in fiber composition with training, but instead, found an increase in the oxidative capacity of all the fiber types with endurance exercise (Gollnick et al., 1973; Costill et al., 1976; Thorstensson et al., 1976; Uehara et al., 1985; Hodgson et al., 1985). Generally, in human athletics, high repetition, low intensity exercise relies on the recruitment of slow twitch fibers and results in improved oxidative capacity of the fibers, while low repetition, high intensity exercise causes recruitment and hypertrophy of the fast twitch fibers (Brooks and Fahey, 1984). Similarly, Lindholm (1979) observed that in Standardbreds, SO fibers were activated at lower work levels, and with increasing speed, FOG and FG fibers were selectively recruited. According to Prince et al. (1976), fast twitch fibers may have more potential to change in oxidative capacity and size due to exercise than slow twitch fibers.

Researchers have tried to determine if there is a relationship between specific types of training and muscle fiber types in human athletes. Burke et al. (1977) found no difference in fiber types between male and female cyclists, and also found no correlation between fiber type and success in competitive cycling. Costill et al. (1976) studied

muscle fibers of track athletes, and while they found no differences between males and females, they did find a large difference in fiber size and composition between javelin throwers and endurance athletes. This led them to surmise that athletic success may be due, in part, to muscle fiber composition. Other research supports these findings, and it can be concluded that endurance athletes have a lower percentage of fast twitch fibers than do athletes involved in anaerobic-type events (Prince et al., 1976; Thorstensson et al., 1976; Brooks and Fahey, 1984). The same is true in horses. Stull and Albert (1981) looked at the percentage of each muscle fiber type from the biceps femoris and triceps brachii in Belgians, Thoroughbreds, Standardbreds, Quarter Horses and Welsh ponies. They found that while intermediate fibers were the most numerous for all breeds, Thoroughbreds had the most red fibers and Belgians had the most white fibers. Also, Snow and Guy (1976) found a trend for a lower percentage of fast twitch fibers in heavy hunters as compared to Thoroughbreds.

Experimentation with cross innervation of muscle fibers (Romanul, 1971) has shown that the biochemical and contractile properties of fast twitch fibers can be produced in slow twitch fibers, and vice versa. Thus, while it is possible for fibers to change characteristics, it is questionable as to whether or not conditioning can bring about these changes. Many researchers, in fact, have tried

to determine this. Thorstensson et al. (1976) found no change in muscle fiber size or composition in the vastus lateralis after an 8 wk strength training program by 14 males. Similarly, Gollnick et al. (1973) found no changes in percent fast or slow twitch fibers in the vastus lateralis of 6 males following 5 mo of cycling, but they did note that the slow twitch fibers were larger after the training program. In equine research, Guy and Snow (1977) found a significant increase in FOG fibers and a decrease in both FG and SO fibers from 6 different muscles in Thoroughbreds and heavy hunters after 10 wk of conditioning. An increase in FOG fibers due to training was also reported by Nimmo et al. (1982), Uehara et al. (1985) and Hodgson et al. (1985). However, Sigler (1981) found no significant change in fiber type of the biceps femoris of Quarter Horse mares after 28 d of galloping, but did see a trend toward increased SO percentage. In addition, there was no change in muscle fiber diameter of FOG, FG and SO fibers following training. Finally, Freeman et al. (1985a) found no change in percentage distribution of each fiber type from the biceps femoris of 4 Quarter Horse geldings following 135 d of low intensity galloping, but did note a significant increase in SO fiber diameter, thus indicating that this type of endurance training could increase oxidative fiber diameter.

Protein Function and Usage

During exercise the working muscles require substrates for energy metabolism. The substrate chosen depends on the duration of work, the intensity of work, the variation in size of workload and the diet the individual is receiving (Hultman, 1967). Generally, the substrate used is carbohydrate or lipid, but protein also is used to a limited extent in energy metabolism (Brooks and Fahey, 1984). In addition to this, protein has many other structural and metabolic functions in the body. As Brooks and Fahey (1984) point out, proteins are assemblages of individual amino acids. Because proteins are vital constituents of the nucleus and protoplasm of every cell (Bogert et al., 1973), and are the main constituents of body organs and tissues (Cunha, 1980), they are essential for life. In the horse, protein comprises 22% of the fat-free body (Cunha, 1980) and has many important functions. First of all, it is utilized in building and maintaining structural units of the body. These structural units include bone, cartilage, connective tissue, blood vessels, muscle, hair, hooves and blood cells. Secondly, protein is utilized in the formation of enzymes, hormones, immune antibodies and hereditary material (Cunha, 1980; Brooks and Fahey, 1984). Finally, a small portion of dietary protein is used as an energy source, particularly when fats and carbohydrates are in short supply (Stegemann, 1981). In addition to this, proteins may give rise to

glucose through gluconeogenesis and support the use of other fuels, such as fat (Brooks and Fahey, 1984).

In both the human and the horse, skeletal muscle, which makes up about 40 to 50% of the body (Hooks, 1974), is comprised of approximately 75% water and 16 to 22% protein (Karpovich, 1965; Forrest et al., 1975). Based on their solubilities, muscle proteins are categorized as sarcoplasmic, myofibrillar, or stromal. The sarcoplasmic proteins include myoglobin, hemoglobin and enzymes associated with the citric acid cycle, the electron transport chain and glycolysis. The myofibrillar proteins include actin, myosin, troponin, tropomyosin, alpha- and beta-actinin, c-protein and m-proteins, and are associated with muscle contraction. Finally, the stromal proteins are found in connective tissue (Forrest et al., 1975).

Research has shown that exercise can induce protein catabolism (Mole and Johnson, 1971). Butterly and Lindsay (1974) have suggested that even at nitrogenous equilibrium there is a large turnover of skeletal muscle protein and evidence of substantial exchange between muscle and plasma free amino acids. Similarly, Laurent and Millward (1980) found that both protein synthesis and degradation increase during muscle hypertrophy in chickens. As a result of the continual utilization of protein for tissue building or repair, an adequate supply of dietary protein is essential.

Protein Metabolism in Response to Exercise

Since protein plays an important and essential role during exercise, equine researchers have tried to determine whether the working horse requires higher levels of dietary protein than the sedentary horse. This issue remains quite controversial. Generally, equine exercise physiologists can look to human exercise physiology research for answers to their questions, yet only a limited amount of research has been done on protein metabolism in human athletes. In fact, as Astrand and Rodahl (1977) point out, the exact protein requirements for different categories of athletes have not yet been established by scientifically controlled metabolic balance studies. Still, some studies aimed at determining the relationship between protein intake and exercise in the human athlete have been conducted, although the results are somewhat inconclusive. The Food and Agriculture Organization of the United Nations (1962) suggested that increased protein intake might be necessary if heavy muscular work is being performed. Similarly, Briggs and Calloway (1979) state that since exercise tends to result in increased muscle size and strength, an athlete might require additional dietary protein to assist in the building of muscle tissue. Bogert et al. (1973), however, have observed that while muscular work is the largest single factor in determining energy needs, it has no appreciable effect on protein requirements except during initial periods

of training when muscular tissue is developing. Gontzea et al. (1975) report similar findings, and suggest that protein intake should be increased for a few days following the initiation of a training program or an increase in training intensity. Finally, Brooks and Fahey (1984) point out that high resistance trained athletes may positively affect lean body mass by consuming high-protein, high-calorie diets.

All researchers, however, do not feel that additional dietary protein is needed to enhance athletic performance or promote the development of muscle tissue. Rasch and Pierson (1962) found that protein supplements did not increase body weight, muscle strength or muscle hypertrophy in college males doing weight training. Furthermore, Crampton (1964) reported that increasing protein above maintenance level as work increases had no beneficial effect on performance or fitness. Also, Consalazio et al. (1975) found that while men on high protein diets did experience an increase in muscle mass and body protein stores, the additional body protein did not enhance exercise performance.

It must be noted that exercise induced protein deficiencies are rather rare in human and equine athletics (Crampton, 1964; Mole and Johnson, 1971; Meyer, 1980). As Bogert et al. (1973) point out, athletes require additional carbohydrates and fat in their diet to provide necessary energy for exercise. Since protein is an integral part of many foods, protein intake generally increases with

increased total food consumption. The same holds true for equine athletes. As Hinkle et al. (1981) point out, many horse producers routinely feed much higher levels of protein to working horses than what is required for maintenance. In a survey conducted by Winter and Hintz (1981), it was found that due to increased energy intake, Thoroughbred race horses consumed approximately 2 1/2 times more crude protein than the NRC (1978) recommends. According to Cunha (1980), most excess protein is deaminated through the urea cycle and excreted, while some serves as an energy source or is converted to and stored as fat through complex body mechanisms. However, the overfeeding of protein is very costly and may even be detrimental. Slade et al. (1975) observed profuse sweating as well as increased heart and respiration rates in endurance trained horses fed a diet containing 10.8% digestible protein, or approximately 17.6% crude protein.

Protein status of the animal body is generally determined through the use of nitrogen balance trials. These trials indicate whether the amount of protein metabolized in the body is equal to, less than, or greater than the amount of protein in the feed (Bogert et al., 1973; Brooks and Fahey, 1984). Urine and feces are collected and analyzed for nitrogen content. Fecal and urinary nitrogen are added together and subtracted from the nitrogen content of the feed to determine nitrogen status. As Bogert et al.

(1973) point out, when intake and output of nitrogen are approximately equal, the body is in a state of nitrogen equilibrium. A positive nitrogen balance occurs when intake is greater than output, and indicates that new tissue is being synthesized with consequent nitrogen retention. Lin and Huang (1982) and Oddoye and Margen (1979) point out that nitrogen deposition does occur when protein intake is very high. Several other researchers have also found that high levels of protein intake resulted in increased nitrogen retention (Slade et al., 1970; Harper and Vander Noot, 1974; Consalazio et al., 1975; Hinkle et al., 1981; Freeman et al., 1985b). Finally, a negative nitrogen balance results if output of nitrogen is greater than intake. This can occur if protein intake is less than that needed to maintain body tissues or if the body must burn protein because the diet fails to provide sufficient carbohydrate and fat to meet the energy needs. A prolonged negative nitrogen balance is undesirable since it is detrimental to growth and(or) performance (Bogert et al., 1973). However, Consalazio et al. (1975) found that individuals consuming the recommended amount of dietary protein went into negative nitrogen balance when they began a weight training program. Within a few days to a week, however, nitrogen balance was restored.

One factor which has concerned researchers is the loss of nitrogenous compounds in sweat during exercise. Bogert

et al. (1973) and Brooks and Fahey (1984) point out that nitrogenous compounds are lost not only in urine and feces, but also in sweat, semen, menses, phlegm, hair and nail clippings. These nitrogenous losses are often not accounted for in nitrogen balance studies. While some researchers feel that nitrogen losses in the sweat are low and have little effect on nitrogen balance (Bogert et al., 1973; Meyer, 1980), others feel that a significant amount of nitrogen may be lost, especially when sweating is profuse. Consalazio et al. (1975) believe that the loss of nutrients through sweat is relevant in determining requirements, particularly when sweat rates are increased, and that nutrient losses during profuse sweating could decrease the accuracy of human metabolic balance studies. Similarly, Calloway et al. (1971) conducted nitrogen balance studies in sedentary and exercising men and found that values based only on urine and fecal collections were 45% lower than when nitrogen lost in sweat, desquamated cells, nails and hair was accounted for. It is known that the horse has an extremely high sweat protein concentration in comparison with other species (Jenkinson et al., 1974; Kerr et al., 1983), and this may have some effect on nitrogen requirements. Freeman et al. (1985a) examined water turnover rates and urinary output in exercising stock-type geldings, and found that large amounts of water (and perhaps nitrogen) are lost through the sweat. Finally, while Hintz

(1982) and Slade (1979) realize that nitrogen containing material is lost through sweat in the working horse, they point out that the increased feed intake needed to supply energy for work generally provides more than an adequate amount of dietary protein.

In the mature horse the recommended level of dietary protein intake, regardless of exercise level, is 8.5% crude protein/d (NRC, 1978). This recommendation is based primarily on research done with endurance trained horses (Slade et al., 1975) and draft horses (Harvey et al., 1939). As previously explained, Slade et al. (1975) found that endurance trained horses fed a diet containing twice the recommended level of dietary protein exhibited profuse sweating and increased heart and respiration rates following a 35 to 40 mile endurance ride. When compared to results obtained on lower protein diets, it was concluded that the high protein diet was the least effective in developing stamina. Similarly, Harvey et al. (1939) found that two draft geldings maintained a positive nitrogen balance when fed a limited amount of timothy hay and oats, even when doing heavy work. Thus, it was concluded that the working horse required no additional dietary protein above the maintenance level. It should be noted, however, that no assessments of lean body mass were made (such as muscle biopsies) at any time during the 11 wk trial. Since both horses lost weight during the study, it is possible that

this loss may, in part, have been at the expense of muscle protein.

In more recent years, additional studies concerning the protein needs of exercising horses have been conducted, and many support the NRC recommendations. Hintz et al. (1980) found that feeding higher levels of dietary protein (diets containing 12% and 24% crude protein) had no effect on the performance of endurance horses, but the high protein diets were quite costly and led to a slight decrease in feed efficiency. Thus, the feeding of additional dietary protein was not recommended. In another study, 18 mature horses were used to determine dietary crude protein requirements for different levels of activity (Patterson et al., 1985). Three levels of crude protein (5.5, 7.0 and 8.5%) and three levels of physical activity (maintenance, medium work and intense work) were examined in a 3 x 3 factorial design. It was determined that 1.9 g D.P./w^{.75} was an adequate minimum protein requirement for maintaining blood proteins in the working horse, regardless of workload, provided the horse's digestible energy needs were met. This is much lower than the NRC (1978) recommendation of 2.7 g D.P./w^{.75}, but is similar to that of Harper and Vander Noot (1974), who determined that 2.08 g D.P./w^{.75} was adequate for the non-exercising horse.

All research, however, does not support the NRC protein recommendations. Freeman et al. (1981) and Hinkle et al.

(1981) found that conditioning tended to increase nitrogen retention in mature stock-type geldings, as did increasing nitrogen intake. In a follow-up study, Freeman et al. (1985a) found that in response to long term, low intensity exercise (135 d at a workload of 2800 kg-km/d and a galloping speed of .45 km/min), mature horses tended to experience an increase in nitrogen balance and changes in skeletal muscle metabolism, thus suggesting that protein turnover may be altered due to exercise. In another study, Freeman et al. (1985b) examined the effects of varying levels of nitrogen intake on nitrogen metabolism in the physically conditioned horse. Isocaloric rations, which contained 7.6, 9.6, 11.6 and 13.6% crude protein, were fed to horses being exercised as outlined above. Results suggested that increasing nitrogen intake tended to cause an increase in nitrogen retention; however, a significant increase in nitrogen balance was only observed when comparing the 7.6% crude protein diet to the 13.6% crude protein diet. Increased nitrogen balance resulting from increased nitrogen intake was demonstrated by Slade et al. (1970) in unconditioned horses as well. However, Freeman et al. (1985b) found that there was a greater rate of increase in nitrogen balance with each unit of increase in nitrogen intake in the conditioned horse. Finally, a significant increase in protein content of the biceps femoris muscle was noted when comparing the 9.6% crude protein diet to the

13.6% crude protein diet, and it was inferred that this response might explain, in part, highly positive nitrogen balance values in working horses consuming diets with constant protein to calorie ratios.

Chapter 3

NITROGEN UTILIZATION AND METABOLIC RESPONSES OF PONIES TO INTENSE ANAEROBIC EXERCISE

ABSTRACT

Eight mature pony geldings with an average weight of 161 kg were randomly assigned to either a control (no exercise) or a treatment group to examine the effects of intense anaerobic exercise on metabolic responses to exercise tolerance tests on the treadmill, muscle fiber type and nitrogen utilization. Treated ponies were worked by pulling a weighted sled for 10 min/d, 5 d/wk for 6 wk. Total weight pulled ranged from 79 to 147 kg and was adjusted daily if necessary to maintain heart rates of 170 to 190 beats/min throughout each 10 min work session. All ponies were subjected to 30 min exercise tolerance treadmill tests on days 0, 21 and 42 of conditioning. There were no treatment differences in the metabolic parameters measured (heart rate, respiration rate, blood lactate concentration and rectal temperature). Since no treatment differences were observed in any of the parameters measured, an exercise tolerance test on the treadmill may not be an appropriate method for evaluating fitness in anaerobically trained ponies. Muscle biopsies were taken from the left biceps

femoris muscle of all ponies at the end of the 6 wk training period, and fiber type was determined. There was no treatment difference in percent FG; and percent FOG, however, percent SO was significantly lower in the exercised group. Six day nitrogen balance trials were conducted on both groups prior to conditioning and on days 1 to 6 and 37 to 42 of conditioning. There were no significant treatment differences in fecal and urinary nitrogen excretion or nitrogen balance. In the third period nitrogen absorbed was higher ($P < .05$) in the exercised group than in the control group, however nitrogen intake tended to be higher in the exercised group. When analyzed relative to intake, though, there was no period or treatment difference in nitrogen balance or nitrogen absorbed. Nitrogen intake was highly correlated with fecal and urinary nitrogen excretion, nitrogen balance and nitrogen absorbed. It appears, then, that nitrogen balance is affected more by nitrogen intake than by anaerobic exercise training.

INTRODUCTION

A number of horses are produced for ultimate use in performance events, therefore an understanding of the relationship of nutrition to exercise is important. Cardiovascular fitness is enhanced through various types of conditioning programs (Sigler, 1981; Erickson et al., 1985; Sexton et al., 1985) and muscle fiber characteristics may

also be affected by training (Guy and Snow, 1977; Hodgson et al., 1985; Uehara et al., 1985). Generally, as the intensity or duration of exercise increases, the energy requirements of the horse increase as well (NRC, 1978). A more controversial point is whether or not the horse's protein requirements increase as a result of increasing workload. The National Research Council (NRC, 1978) maintains that the mature working horse requires no additional dietary crude protein above the maintenance level of 8.5% crude protein/d. This claim is supported by work done in draft horses (Harvey et al., 1939) and light horses (Patterson et al., 1985). However, studies conducted by Freeman et al. (1985a) indicate that nitrogen retention may be increased in horses performing long term, low intensity exercise, and that protein turnover may be altered. The objectives of this study, then, were to investigate the effect of intense anaerobic exercise on nitrogen utilization in ponies and to determine cardiovascular and muscular adaptations to this exercise.

EXPERIMENTAL PROCEDURES

Experimental Animals

Eight mature pony geldings, ranging in age from 3 to 11 yr, with an average weight of 161 ± 9.8 kg (Appendix A) were randomly assigned to either control (no exercise) or

treatment groups to determine the effects of intense anaerobic-type work on metabolic responses to a standard exercise tolerance treadmill test, as well as on muscle fiber type and nitrogen utilization. Ponies were housed individually in 3 x 7 m pens, except during collection periods, at which times they were tied to prevent coprophagy and to keep movement to a minimum. Treated ponies were worked by pulling a weighted sled (79 to 147 kg of total weight pulled) across a gravel surface for 10 min/d, 5 d/wk for 6 wk. Heart rate was measured continuously throughout each work session by a portable heart rate monitor (EQB, Unionville, PA). Weight pulled was adjusted daily to maintain a heart rate of 170 to 190 beats/min throughout each 10 min work session (Appendix B).

Feeding and Sample Collection

A complete pelleted diet (table 1) containing 13.6% crude protein (100% dry matter basis) was fed to all ponies. Ponies were weighed twice weekly and were fed at levels to maintain zero body weight change throughout the trial. Chromic oxide was used as an external indicator and incorporated into the pellets at a level of .24%. Chemical analysis of the chromic oxide diet is shown in table 2. Ponies were fed twice daily at 12 h intervals and were allowed ad libitum access to water. Following precollection adjustment periods of 10 d, 6 d nitrogen balance trials were

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Ingredient	%	
	Base Diet	Chromic Oxide Diet
Corn	48	47
Sun cured alfalfa	46	46
Molasses, liquid	5	5
Dicalcium phosphate	.5	.5
Trace mineral salt ^a	.5	.5
Chromic oxide	-	.24
Diluent ^b	-	1

^a salt, iron oxide, iron carbonate, manganese oxide, magnesium oxide, copper oxide, cobalt carbonate, zinc oxide, calcium iodate, calcium carbonate, calcium stearate, propylene glycol, mineral oil.

^b ground corn.

TABLE 2. CHEMICAL ANALYSIS OF CHROMIC OXIDE DIET ON 100%
 DRY MATTER BASIS

Dry matter, %	85.80
Ash, %	8.43
Crude protein, %	13.56
Nitrogen, mg/g	21.70
Gross energy, Mcal/kg	4.51
Ether extract, %	2.16
Crude fiber, %	15.93
NFE, %	54.84
Calcium, %	.98
Phosphorus, %	.46

conducted on both groups prior to conditioning, at 1 to 6 d of conditioning, and at 37 to 42 d of conditioning. Daily feed samples were stored in plastic freezer bags and saved for later analysis.

Fecal grab samples were collected once daily for 6 d to represent each 2 h interval after feeding. Samples were stored in plastic bags and frozen for later analyses of chromium, calcium, phosphorus and proximate components.

Total urine was collected for 5 d in urine harnesses according to methods described by Haley (1981). Urine harnesses were washed daily to help minimize urease activity. Urine was collected and measured every 3 h and a 1% subsample was obtained. Each sample was acidified with hydrochloric acid and was then frozen in an airtight container for later analyses of nitrogen, calcium and phosphorus. Ponies were tied and remained standing throughout collection periods to prevent urine spillage and coprophagy. They were hand-walked for 15 to 20 min/d to prevent stiffness.

Preparation of Samples and Laboratory Analysis

Individual fecal samples were dried at 50 C in a draft oven and were then composited on an equal weight basis into one sample per pony per period. Feed samples were composited in a similar manner. Both feed and fecal samples were ground through a 1 mm screen in a Wiley Mill (models

no. 1 and 3, Arthur H. Thomas Co., Philadelphia, PA) and stored for analyses in airtight containers.

Dry matter content was determined gravimetrically after drying in a vacuum oven at 102 C for 24 h.

Chromium content was determined by wet ashing followed by atomic absorption spectroscopy (AOAC, 1984).

Ether extract content was determined gravimetrically following 4 h of refluxing and 1 h of drying in a vacuum oven as outlined by the AOAC (1984).

To determine crude fiber content, samples were treated with a series of acid and alkaline washes, dried in a vacuum oven at 102 C and ashed for 1 h in a muffle furnace at 550 C. Mineral matter also was determined by ashing samples in a muffle furnace (AOAC, 1984). Following ashing, calcium content was determined by atomic absorption spectroscopy and phosphorus content was determined colorimetrically according to AOAC (1984) procedures.

Energy content of feed and fecal samples was determined by oxygen bomb calorimetry (AOAC, 1984).

The kjeldahl method of acid digestion followed by titration was used to determine the crude protein content of all samples. AOAC (1984) procedures were followed for the acid digestion, however boric acid was used in the distillation.

Samples were analyzed in quadruplicate for all constituents measured. Digestion coefficients were

determined for the parameters measured, and balance values were calculated for nitrogen, calcium and phosphorus. Nitrogen absorption was also calculated.

Standard Exercise Tolerance Test

All ponies were subjected to an exercise tolerance treadmill test similar to that described by Sexton et al. (1985) on days 0, 21 and 42 of conditioning. The step-wise test consisted of working the ponies on an equine treadmill (Anamill, MLR Design, Sundusky, OH) at a 7 degree incline for 30 min. Speed was increased every 5 min as follows: .8 m/s, 1.0 m/s, 1.6 m/s, 2.2 m/s, 2.8 m/s and 3.4 m/s.

Heart rates were measured with a portable heart rate monitor (EQB, Unionville, PA) at rest off the treadmill, at rest on the treadmill, at every 5 min interval during the 30 min test and at 10 min of recovery.

Respiration rates were measured at rest and immediately following completion of the test by observing the ponies' flanks and nostrils.

Rectal temperatures were taken at rest and after completion of the test by a rectal thermometer.

Venous blood samples (approximately 20 ml) were taken with a needle and syringe at rest and immediately following the treadmill test for determination of lactic acid. Samples were placed in ice, and heparin was used to prevent clotting. Following centrifugation, lactic acid content of

each sample was determined by a YSI 23L Lactate Analyzer (Yellow Springs Instrumentation Co., Yellow Springs, OH).

Muscle Biopsies

Following the 6 wk conditioning period, muscle biopsies were taken from the left biceps femoris muscle of each pony. Ponies were prepared for the biopsy by tranquilizing intravenously with .5 ml of Rompun/100 lb of body weight (Mobay Corp., Animal Health Division, Shawnee, KS), clipping the area, and using 2.5 to 7.5 ml of Lidocaine (TechAmerica Group Inc., Elwood, KS) as a local anesthetic. The biopsy area was disinfected with Betadine scrub, and a scalpel blade was used to make a small incision in the medial portion of the biceps femoris. A biopsy needle, 4 cm in diameter, (Stille, Sweden) similar to that described by May (1981), was inserted into the muscle and a sample was obtained. Each sample was placed on a cork with Tissue-Tek-II O.C.T. compound, frozen immediately in liquid nitrogen and stored in an ultra-low freezer at -65 C until preparation for analysis.

To determine muscle fiber type, frozen serial sections of each sample were cut (8 um thick) at -18 C with a Slee cryostat and microtome (Slee International, Inc., Tiverton, RI) and were placed on coverslips or slides. Muscle sections were then stained for actomyosin ATPase activity as described by Guth and Samaha (1970) (Appendix C) or DPNH-

tetrazolium reductase activity according to Engle and Brooke (1966) (Appendix D).

Stained sections were examined and photomicrographs were taken with a Nikon Microphot-FX microscope. Fibers were then identified by oxidative capacity (red or white) and contraction speed (fast or slow), and were then classified as fast glycolytic (FG), fast oxidative glycolytic (FOG) and slow oxidative (SO) (Brooks and Fahey, 1984).

Statistical Analysis

All data were subjected to analysis of variance procedures using pony, treatment and period as main effects (Ott, 1984). Correlations between nitrogen intake and fecal nitrogen, urinary nitrogen, nitrogen balance and nitrogen absorption were determined, as were correlations between feed intake and proximate component digestibility. Regression analysis was used to determine the relationship between nitrogen balance and nitrogen intake, as well as to determine if there was a difference between groups in heart rate response to each treadmill test.

RESULTS AND DISCUSSION

Heart Rate Response to Exercise Tolerance Tests

Heart rate responses (beats/min) to the exercise

tolerance treadmill tests in both the control and the anaerobically exercised ponies over the 6 wk training period are shown in table 3. Mean resting heart rate off the treadmill over all test days was 46.2 beats/min for the control group and 46.3 beats/min for the exercised group. There was no change over time in the control group, however, resting heart rate off the treadmill decreased ($P < .05$) from 51.5 beats/min to 39.3 beats/min, from day 0 to day 42 in the exercised group. This agrees with work done by Marshland (1968), who observed reduced resting heart rates following endurance training in Standardbred trotters. Nonsignificant trends toward lower resting heart rates following conditioning programs were also reported by Stewart (1972), Milne et al. (1977) and Burke et al. (1981). Resting heart rate on the treadmill was slightly higher than off the treadmill, and averaged 49.0 beats/min for the control group and 50.8 beats/min for the exercised group over all test days. These values are similar to those reported by Pearson (1980), Erickson et al. (1985) and Miller et al. (1985) in the Quarter Horse. This increase in resting heart rate on the treadmill was also observed by Pearson (1980), and is similar to the anticipatory response shown in race horses prior to exercise (Witherington, 1971). Resting heart rate on the treadmill did not differ between groups within day of test or between days of test within groups. This is in agreement with data from other

TABLE 3. MEAN HEART RATE RESPONSE (BEATS/MINUTE) TO EXERCISE TOLERANCE TREADMILL TESTS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Time	Treatment						SE ^a
	Control			Exercise			
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42	
Rest off treadmill	44.8 ^{bc}	49.5 ^{bc}	44.3 ^{bc}	51.5 ^c	48.3 ^{bc}	39.3 ^b	4.0
Rest on treadmill	49.8	47.8	49.5	53.8	54.0	44.5	5.2
5 min	103.5 ^d	80.8 ^b	79.3 ^b	93.8 ^{cd}	83.8 ^{bc}	97.3 ^d	4.0
10 min	102.3 ^{bc}	95.3 ^b	99.5 ^{bc}	112.5 ^{cd}	113.8 ^{cd}	122.3 ^d	4.8
15 min	124.5 ^{bc}	110.8 ^b	126.3 ^{bc}	143.0 ^c	124.5 ^{bc}	137.8 ^c	7.5
20 min	153.0	157.0	157.5	165.8	166.8	157.5	7.2
25 min	189.3	170.8	193.5	186.8	184.8	169.3	12.0
30 min	211.3	200.5 ^e	208.8	213.8	206.8	205.0	7.6
10 min recovery	109.3	91.0 ^f	89.5	95.8	91.8	94.8	9.2

^aStandard error of means.

^{b,c,d}Means in the same row with different superscripts differ significantly (P<.05).

^eSE = 9.3.

^fSE = 11.3.

researchers which indicates that resting heart rate on the treadmill is not decreased with training (Pearson, 1980; Sigler, 1981; Erickson et al., 1985; Sexton et al., 1985).

Heart rates during the treadmill tests did not vary with conditioning (table 3). Other researchers (Sigler, 1981; Erickson et al., 1985; Sexton et al., 1985) have reported a decrease in exercising heart rate on the treadmill following training, however no such change was observed here. This agrees with work done by Milne et al. (1977) in Standardbreds who observed no decrease in exercising heart rates with training. There was, however, a trend ($P=.18$) for the exercised group to have a lower 25 min heart rate during the third test (169.3 beats/min) than the control group (193.5 beats/min), yet no significant treatment or period differences were observed in 30 min heart rate, which averaged 206.8 beats/min for the control group and 208.5 beats/min for the exercised group over all test days. These ending values are higher than those reported by Sigler (1981) and Erickson et al. (1985) in Quarter Horses, but are similar to values observed by Sexton et al. (1985) in unfit ponies. Perhaps differences were not seen because ponies were nearing maximal heart rates, which are not greatly affected by training (Sexton et al., 1985). Another explanation may be that the long duration treadmill test may not have been an appropriate method for evaluating fitness in anaerobically trained ponies.

Campbell et al. (1985) found that heart rate at the end of a 20 min exercise tolerance treadmill test was not decreased in Quarter Horses after undergoing aerobic exercise (30 min of riding/d) followed by intermittent anaerobic cutting training.

No period or treatment differences were seen in 10 min recovery heart rates, which averaged 96.6 beats/min for the control group and 94.1 beats/min for the exercised group over all test days. These values are similar to those observed by Pearson (1980) in mature Quarter Horses following 30 min treadmill tests at 148 m/min and 172 m/min. Heart rates dropped rapidly during early recovery from 206.8 beats/min to 96.6 beats/min in the control group and from 208.5 beats/min to 94.1 beats/min in the exercised group. This rapid drop in heart rate has been reported by other researchers in Quarter Horses (Pearson, 1980; Sigler, 1981; Erickson et al., 1985) and in ponies (Sexton et al., 1985). Campbell et al. (1985) observed no differences between trained and untrained Quarter Horses in 10 min recovery heart rate response to a cutting standard exercise tolerance test, (3 2.5 min bouts of cutting with 30 s of work, 25 s of rest, 30 s of work, 25 s of rest and 40 s of work with 1 min rest intervals between bouts). While Sigler (1981) found a decrease in 10 min recovery heart rate in Quarter Horse mares after 28 d of gallop training, no differences were apparent in 30 min recovery heart rate. This agrees with

work done by Littlewort and Hickman (1969) and Milne et al. (1977) which indicates that recovery heart rates are not good indicators of fitness in the horse. While Cardinet et al. (1963) and Stewart (1972) indicated that recovery of heart rate following exercise appeared to take longer in unfit horses, Witherington (1971) and Brooks and Fahey (1984) point out that recovery of heart rate is dependent on many factors including maximum heart rate, intensity and duration of exercise and climatic conditions during exercise.

To determine heart rate response to the treadmill test, heart rates during exercise and recovery were analyzed over time within the same treatment and test day (table 4). Quadratic regression analysis was conducted, and it was determined that there was no treatment difference in heart rate response to the treadmill tests. To summarize the response over both groups and all test days, heart rate at 15 min of exercise was higher ($P < .05$) than at rest, heart rate at 30 min of exercise was higher than at 15 min, and heart rate at 10 min of recovery was lower than at 30 min of exercise. An increase in heart rate with increasing levels of exercise has been reported by other researchers (Thomas and Fregin, 1981; Erickson et al., 1985; Sexton et al., 1985).

TABLE 4. DIFFERENCES IN HEART RATE RESPONSE (BEATS/MINUTE) TO EXERCISE TOLERANCE TREADMILL TESTS WITHIN EACH TREATMENT AND TEST DAY

Time	Treatment					
	Control			Exercise		
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42
Rest off treadmill	44.8 ^a	49.5 ^a	44.3 ^a	51.5 ^a	48.3 ^a	39.3 ^a
Rest on treadmill	49.8 ^a	47.8 ^a	49.5 ^a	53.8 ^a	54.0 ^a	44.5 ^a
5 min	103.5 ^b	80.8 ^b	79.3 ^b	93.8 ^b	83.8 ^b	97.3 ^b
10 min	102.3 ^b	95.3 ^{bc}	99.5 ^c	112.5 ^b	113.8 ^c	122.3 ^c
15 min	124.5 ^{bc}	110.8 ^c	126.3 ^d	143.0 ^c	124.5 ^c	137.8 ^c
20 min	153.0 ^c	157.0 ^d	157.5 ^e	165.8 ^{cd}	166.8 ^d	157.5 ^d
25 min	189.3 ^d	170.8 ^d	193.5 ^f	186.8 ^{de}	184.8 ^e	169.3 ^d
30 min	211.3 ^d	200.5 ^{eg}	208.8 ^f	213.8 ^e	206.8 ^f	205.0 ^e
10 min recovery	109.3 ^b	91.0 ^{bcg}	89.5 ^{bc}	95.8 ^b	91.8 ^b	94.8 ^b
SE ^h	10.1	8.5	6.6	9.8	5.4	6.5

a,b,c,d,e,f Means in the same column with different superscripts differ significantly (P<.05).

^gSE = 9.9.

^hStandard error of means within each treatment and test day.

Respiration Rate Response to Exercise Tolerance Tests

Respiration rates (exhalations/min) before and after the exercise tolerance treadmill tests in both the control and the anaerobically exercised ponies over the 6 wk training period are shown in table 5. Pre-test respiration did not differ with test day or treatment, and averaged 16.8 exhalations/min for the control group and 17.3 exhalations/min for the exercised group. These values are numerically lower than those reported by Campbell et al. (1985) and Topliff et al. (1985), but are similar to those reported by Pearson (1980), Sigler (1981) and Moffitt et al. (1985) in the Quarter Horse. No test day or treatment effects were seen in post-test respiration rates, and they averaged 126 exhalations/min for the control group and 141.7 exhalations/min for the exercised group. These values are similar to those reported by Pearson (1980) and Moffitt et al. (1985). Snow and MacKenzie (1977a) and Sigler (1981) also found no significant changes in respiration rates as a result of conditioning in Standardbreds and Quarter Horses. Campbell et al. (1985), however, did see a significant decrease in respiration rates at the end of a 20 min treadmill test following aerobic and intermittent anaerobic cutting training in Quarter Horses.

Post-test respiration rates were higher ($P < .05$) than pre-test values over all test days and treatments. This increase in respiration rate due to exercise has been

TABLE 5. MEAN RESPIRATION RESPONSE (EXHALATIONS/MINUTE) TO EXERCISE TOLERANCE TREADMILL TESTS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42	
Pre-test respiration	25.0	13.5	12.0	22.0	14.0	16.0	4.9
Post-test respiration	123.0	103.8 ^b	151.5	131.5	150.5	143.0	12.2

^aStandard error of means.

^bSE = 19.3.

reported by other researchers (Pearson, 1980; Sigler, 1981; Moffitt et al., 1985). Pearson (1980) found that increasing treadmill speed resulted in higher post-test respiration rates. Similarly, Thomas and Fregin (1981) found that oxygen consumption in Morgan and Thoroughbred mares increased linearly with increasing treadmill speed.

Considerable individual variation in respiration rate was observed both prior to and following the treadmill tests. Other researchers (Cardinet et al., 1963; Stewart, 1972; Kelly, 1977; Sigler, 1981) observed similar effects, therefore respiration rate is not generally believed to be a reliable indicator of fitness.

Rectal Temperature Response to Exercise Tolerance Tests

Rectal temperatures before and after the exercise tolerance treadmill tests in both the control and the anaerobically exercised ponies over the 6 wk training period are shown in table 6. Pre-test temperatures did not differ with test day or treatment, and averaged 38.7 C for the control group and 38.6 C for the exercised group. These values are similar to those reported by Pearson (1980) and Erickson et al. (1985) in the Quarter Horse and Sexton et al. (1985) in ponies.

Post-test rectal temperatures which averaged 40.9 C and 40.5 C for the control and exercised groups, respectively, also did not differ with test day or treatment. Post-test

TABLE 6. MEAN RECTAL TEMPERATURE RESPONSE (C) TO EXERCISE TOLERANCE TREADMILL TESTS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42	
Pre-test temperature	38.8	38.8	38.4	38.6	38.6	38.6	.25
Post-test temperature	41.0 ^{bc}	41.1 ^{cd}	40.4 ^{bc}	40.5 ^{bc}	40.8 ^{bc}	40.3 ^b	.27

^aStandard error of means.

^{b,c}Means in the same row with different superscripts differ significantly (P<.05).

^dSE = .32.

values are similar to those recorded by Lindholm and Saltin (1974) in Standardbreds after various trotting tests and Pearson (1980) in Quarter Horses following an exercise tolerance treadmill test. While no treatment differences were seen in post-test rectal temperature, significant decreases in arterial blood temperature due to conditioning at each step of an exercise tolerance treadmill test were reported by Bayly et al. (1983) and Erickson et al. (1985) in Quarter Horses and Sexton et al. (1985) in ponies.

Post-test temperatures averaged 2.1 C higher ($P < .05$) than pre-test values over all test days and treatments. Increases in either blood or rectal temperatures due to exercise have been noted by other researchers in Standardbreds (Lindholm and Saltin, 1974), Quarter Horses (Pearson, 1980; Erickson et al., 1985) and ponies (Sexton et al., 1985)

Lindholm and Saltin (1974) found that in Standardbreds, muscle temperature during exercise was higher than rectal temperature, and Bayly et al. (1983) found that maximal rectal temperature was actually not attained until 5 min post-exercise. Furthermore, Forster et al. (1985) found that arterial blood temperature was significantly higher than rectal temperature during exercise in ponies, and concluded that blood temperature better reflected true core temperature than rectal temperature.

Blood Lactic Acid Response to Exercise Tolerance Tests

Blood lactic acid levels (mmol/liter) before and after the exercise tolerance treadmill tests in both the control and the anaerobically exercised ponies over the 6 wk training period are shown in table 7. Pre-test lactate did not differ significantly with test day or treatment, and averaged 1.0 mmol/liter for the control group and .98 mmol/liter for the exercised group over all days. These values are similar to those reported by other researchers (Lindholm and Saltin, 1974; Mullen et al., 1979; Erickson et al., 1985; Sexton et al., 1985) who also concluded that resting blood lactate levels were not affected by conditioning.

Blood lactate concentration following the exercise tolerance treadmill tests also did not differ with test day or treatment, and averaged 14.4 mmol/liter for the control group and 13.3 mmol/liter for the exercised group over all test days. Several researchers have observed a decrease in exercising or post-exercise blood lactate levels as a result of conditioning (Pearson, 1980; Sigler, 1981; Erickson et al., 1985; Sexton et al., 1985; Shelle et al., 1985). However, Snow and MacKenzie (1977b) observed no change in lactate response following a cantering session in Thoroughbreds and heavy hunters after 10 wk of conditioning. Similarly, Topliff et al. (1985) found no significant change in blood lactate concentration following an anaerobic

TABLE 7. MEAN BLOOD LACTATE RESPONSE (MMOL/LITER) TO EXERCISE TOLERANCE TREADMILL TESTS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42	
Pre-test blood lactate	1.0	1.4	.6	.9	1.0	1.1	.32
Post-test blood lactate	13.6	14.9 ^b	14.6	13.8	13.3	12.9	.91

^aStandard error of means.

^bSE = 1.12.

pulling test after 28 d of conditioning and a glycogen loading program. Campbell et al. (1985) found no significant difference in blood lactate levels at the end of a 2.5 min exercise tolerance cutting test when comparing pre-training values with those observed after aerobic and intermittent anaerobic cutting training. Finally, while a trend was observed, Rodiek et al. (1982) found no significant differences in lactate concentration following a 9 min treadmill test in Quarter Horse mares after 4 wk of training.

Average post-test blood lactate values of 14.4 mmol/liter for the control group and 13.3 mmol/liter for the exercised group were much higher than those observed in aerobically conditioned Quarter Horses (Pearson, 1980; Sigler, 1981; Erickson et al., 1985) and ponies (Sexton et al., 1985) following a 20 to 30 min exercise tolerance treadmill test. Values were higher than those observed by Campbell et al. (1985) following cutting and treadmill tests in conditioned Quarter Horses, and those reported by Shelle et al. (1985) in conditioned Arabian mares following a treadmill test (22% grade) to fatigue. Values were slightly higher than those reported by Topliff et al. (1985) at the end of an anaerobic pulling test following glycogen repletion in anaerobically trained, glycogen loaded Quarter Horse geldings. It must be noted, however, that at the end of the treadmill tests over all test days and treatments,

heart rates of the ponies exceeded 200 beats/min which is higher than that observed by other researchers in the Quarter Horse (Pearson, 1980; Sigler, 1981; Erickson et al., 1985). The elevated heart rate indicates a greater amount of anaerobic metabolism, and thus a greater accumulation of lactate in the blood. Lindholm and Saltin (1974) reported a blood lactate concentration of 15.7 mmol/liter in fit Standardbred horses at the end of a 2100 m trotting race and approximately 20 mmol/liter after a 6 X 1000 m trotting workout with a mean trotting speed of 11.8 m/s. Similarly, Keenan (1979) observed an average blood lactate concentration of 29.6 mmol/liter 10 min after a 1100 m race in 30 Thoroughbred geldings. Also, Miller et al. (1985) observed blood lactate values of 13.1 mmol/liter in Quarter Horse mares at the end of a treadmill test to exhaustion. Post-test lactate values were higher ($P < .05$) than pre-test values over all test days and treatments. This increase in blood lactate due to a specific exercise bout has been reported by several researchers (Lindholm and Saltin, 1974; Keenan, 1979; Pearson, 1980; Sigler, 1981; Erickson et al., 1985; Moffitt et al., 1985; Sexton et al., 1985; Topliff et al., 1985).

Muscle Fiber Type in Response to Conditioning

Muscle fiber type after the 6 wk conditioning period averaged 27.1% FG, 53.4% FOG and 19.5% SO for the control

group and 34.9% FG, 56.6% FOG, and 8.5% SO for the anaerobically exercised group (table 8). Thus, in the control group, 19.5% of the fibers were slow twitch and 80.5% were fast twitch, while 8.5% were slow twitch and 91.5% were fast twitch in the exercised group. Aside from percent SO fibers in the exercised group, the percentage of each fiber type is similar to that observed by Lindholm (1979) in the gluteus medius muscle of Standardbreds. Values reported by Lindholm were 24% SO (range 16 to 33), 54% FOG (range 35 to 64) and 22% FG (range 7 to 37).

Percentage of each fiber type in the ponies differs somewhat from values reported by other researchers. Sigler (1981) examined muscle fibers from the biceps femoris of trained and untrained Quarter Horses, and reported 14.3% SO, 38.4% FOG and 47.3% FG. Similarly, Kline and Bechtel (1983) examined muscle fibers from the middle gluteus medius of mature and 6 mo old Quarter Horses. No age or sex difference was observed, and fibers averaged 12.7% SO, 43.1% FOG and 44.3% FG. Perhaps the higher percent of FG fibers in Quarter Horses is due to the fact that they are bred more for use in high intensity, short duration events, such as racing, than are ponies.

Stull and Albert (1981) examined muscle fiber type in Thoroughbreds, Standardbreds, Quarter Horses, Belgians and Welsh ponies, and found that there was a significant breed difference between red and white fibers. Both the biceps

TABLE 8. MUSCLE FIBER TYPE AFTER 6 WEEKS OF CONDITIONING IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment		SE ^a
	Control	Exercise	
Fast glycolytic, %	27.1	34.9	6.1
Fast oxidative glycolytic, %	53.4	56.6	8.1
Slow oxidative, %	19.5 ^c	8.5 ^b	3.1

^aStandard error of means.

^{b,c}Means in the same row with different superscripts differ significantly (P<.05).

femoris and the triceps brachii were examined for fiber type, and in the Welsh pony, the percentage of each fiber in the biceps was 14% SO, 66% FOG and 20% FG, while in the triceps it was 13% SO, 57% FOG and 30% FG. There were no significant differences in fiber type between the two muscles examined in any of the five breeds. Values reported for the Welsh ponies, then, are somewhat similar to those observed in the control and exercised ponies in this study.

There was a significant difference in SO fiber percentage between the control and the anaerobically exercised group (table 8). The exercised ponies had less SO fibers ($P < .05$) than the control ponies, and tended to have more FOG and FG fibers. Similarly, Guy and Snow (1977) observed a decrease in SO fibers with 10 wk of mixed endurance and sprint training, and noted a decrease in FG fibers, with a subsequent increase in FOG fiber percentage, apparently due to increased oxidative capacity and contraction speed. Furthermore, Uehara et al. (1985) found that the glycolytic muscle ratio, expressed as a percentage of glycolytic muscle fibers against total number of muscle fibers, was decreased by training in race horses. While Hodgson et al. (1985) observed no change in muscle fiber type over a 7 wk aerobic training program in Standardbred geldings, an increase in the oxidative capacity of the FG fibers was noted. Sigler (1981), however, found no significant changes in fiber type with trot and gallop

training in Quarter Horses, yet a trend ($P=.16$) for an increased percentage of SO fibers was seen after 28 d in the gallop group.

Feed Intake and Nutrient Digestibility

There was no treatment difference in feed intake (table 9), although there was a trend for the control group to be consuming less feed than the exercised group in period 3 ($P=.09$). There was, however, a period difference in intake with both groups consuming less feed ($P<.05$) in the third period than in the first two, since ponies were fed to maintain zero body weight change.

Treatment and period differences were observed in digestible energy and digestible protein intake (table 9). Both groups were consuming less digestible energy ($P<.05$) in the third period than in the first two, however, in the third period the exercised group consumed more ($P<.05$) digestible energy (4.95 Mcal/d) than the control group (3.51 Mcal/d). Similarly, while both groups were consuming less digestible protein in the third period, the exercised group was again consuming more ($P<.05$) (.15 kg/d) than the control group (.12 kg/d).

When digestible energy intake was examined as a percentage of the NRC recommended level, (table 10), there was no treatment difference, but there was a period difference. Both groups were consuming a smaller percentage

TABLE 9. DAILY FEED, DIGESTIBLE ENERGY AND DIGESTIBLE PROTEIN INTAKE (100% DM BASIS) IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						S.E. ^a
	Control			Exercise			
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
Feed intake, kg/d	2.56 ^c	2.65 ^c	1.73 ^b	2.53 ^c	2.57 ^c	2.00 ^b	.11
Digestible energy intake, Mcal/d	7.01 ^d	6.59 ^d	3.51 ^b	6.79 ^d	6.78 ^d	4.95 ^c	.41
Digestible protein intake, kg/d	.20 ^d	.21 ^d	.12 ^b	.20 ^d	.20 ^d	.15 ^c	.01

^aStandard error of means.

^{b,c,d}Means in the same row with different superscripts differ significantly (P<.05).

TABLE 10. DAILY DIGESTIBLE ENERGY AND DIGESTIBLE PROTEIN INTAKE AS A PERCENTAGE OF NRC RECOMMENDED LEVELS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
Digestible energy as percent of NRC, %	99.5 ^d	94.0 ^{cd}	51.3 ^b	95.3 ^{cd}	82.6 ^c	59.3 ^b	4.8
Digestible protein as percent of NRC, %	163.9 ^d	169.2 ^d	100.8 ^b	160.9 ^d	160.3 ^d	122.2 ^c	5.0

^aStandard error of means.

^{b,c,d}Means in the same row with different superscripts differ significantly (P<.05).

of the NRC recommended level in period 3 than in the first 2 periods (51.3% and 59.3% for the control and exercised groups, respectively). While these values appear low, ponies maintained body weight and condition. Webb et al. (1987) found that when fed to maintain body weight and condition score, mature mares undergoing aerobic and cutting training required less digestible energy than the NRC (1978) recommends. Similar findings were reported by Potter et al. (1987) in sedentary Belgian and Percheron horses.

Treatment and period differences were present when digestible protein intake was examined as a percentage of the NRC recommended level (table 10). Percent consumed was lower ($P < .05$) in the third period for both groups, however, in the third period, the exercised group consumed a larger ($P < .05$) percentage of the NRC recommended level (122.2%) than the control group (100.8%).

Digestibilities of proximate components are shown in table 11. There was no treatment difference in dry matter digestibility in the first two periods, however in the third period the exercised group had a significantly higher dry matter digestion coefficient. In both groups dry matter digestibility was lower ($P < .05$) in the third period than in the first period. This is perhaps due to the fact that dry matter digestibility was correlated ($P < .01$) with feed intake, and intake was significantly lower in the third period. Digestibilities for dry matter are slightly lower

TABLE 11. DIGESTIBILITY OF PROXIMATE COMPONENTS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
Dry matter digestibility, %	62.1 ^d	57.8 ^{cd}	45.9 ^b	61.4 ^d	58.5 ^{cd}	54.8 ^c	2.1
Crude fiber digestibility, %	24.1 ^c	8.1 ^c	-30.4 ^b	22.2 ^c	11.3 ^c	.11 ^{bc}	10.2
NFE digestibility, %	76.8 ^c	74.5 ^c	69.2 ^b	76.6 ^c	75.3 ^c	71.3 ^b	1.2
Ether extract digestibility, %	18.3 ^d	7.4 ^{cd}	-6.2 ^b	17.0 ^d	.7 ^{bc}	-3.6 ^{bc}	4.3
Ash digestibility, %	39.7 ^d	41.3 ^d	16.4 ^b	35.9 ^{cd}	39.7 ^d	31.8 ^c	2.4
Crude protein digestibility, %	57.9	57.5	52.6	58.1	55.4	56.3	2.4

^aStandard error of means.

^{b,c,d}Means in the same row with different superscripts differ significantly (P<.05).

than those reported by Hintz and Loy (1966) in Thoroughbred and Quarter Horse fillies being fed a pelleted diet, however, they are within the range of values reported by McNally (1979) in the horse and Reitnour and Salsbury (1976), Reitnour and Treece (1971) and Burch (1982) in the pony.

There were no treatment differences in crude fiber digestibility, but values were highly variable and were biased due to the fact that ponies consumed wood and shavings in their pens immediately prior to collection periods. This explains the low and even highly negative crude fiber digestibilities observed. Parsons (1980) also observed wood chewing in horses receiving a complete pelleted ration, which may indicate a physical need for long stem roughage. Schneider and Flatt (1975) point out, however, that increased fiber intake may decrease the digestibility of other proximate components.

There was no treatment difference in nitrogen free extract (NFE) digestibility, however there was a period difference. NFE digestibility was lower ($P < .05$) in the third period than in the first two periods for both groups. This may be due to the fact that NFE digestibility was correlated ($P < .05$) with feed intake and dry matter digestibility ($P < .001$), but also NFE is determined by subtracting the amount of crude fiber, crude protein, ash and ether extract from 100, and therefore can represent the

errors that are present in the other analyses. Values are similar to those reported by Slade and Hintz (1969) in ponies fed an alfalfa-grain diet, but are slightly higher than those reported by Burch (1982) in ponies and McNally (1979) and Ott (1985) in horses.

Ether extract digestibility was highly variable. While there was no treatment difference, ether extract digestibility was lower ($P < .05$) in the third period than in the first period for both groups. Ether extract digestibility was also correlated with feed intake ($P < .05$) and dry matter digestibility ($P < .01$), however Schneider and Flatt (1975) point out that ether extract digestibility may be biased by ether soluble substances in the feces, and that errors are often present, particularly when fat content of the diet is low. A large variation in ether extract digestibility as well as negative values were also reported by McNally (1979) in grade geldings consuming alfalfa, brome and prairie hay. Similar findings were reported by Ott (1985) in Thoroughbred and Quarter Horse geldings consuming rations of coastal bermudagrass hay or coastal bermudagrass hay plus cracked corn.

Period and treatment differences were seen in ash digestibility. In the control group, ash or mineral digestibility was significantly lower in the third period than in the first two. In the exercised group, the difference was only significant when comparing the second

period with the third. In the third period, ash digestibility was lower ($P < .05$) in the control group than in the exercised group. This difference may be due in part to the fact that ash digestibility is highly correlated ($P < .001$) with feed intake. Aside from the digestibility value during the third period in the control group (16.4%), ash digestibilities were similar to those reported by Burch (1982). Schneider and Flatt (1975) point out, however, that errors may be present in ash digestibility since fecal mineral matter may include large amounts of mineral substances excreted from the blood into the digestive tract, and therefore much of the ash in the feces may not be undigested feed ash.

No period or treatment differences were observed in crude protein digestibility which averaged 56.0% for the control group and 56.6% for the exercised group over all periods. These values are slightly higher than those reported by Burch (1982) in ponies, and are lower than those reported by Hintz and Loy (1966) in horses receiving pelleted diets and Slade and Hintz (1969) and Reitnour and Treece (1971) in ponies receiving mixed diets. Digestibilities observed by these researchers ranged from the low 70's to the mid 80's. Results, however, were similar to those reported by Reitnour and Salsbury (1976), McNally (1979) and Ott (1985).

Calcium and phosphorus balance values are shown in

table 12. All values were negative which was unexpected, since ponies were getting well above the NRC (1978) requirements for calcium and phosphorus. Wood chewing and incomplete availability of calcium and phosphorus in the feed may have been sources of error.

In any digestion trial, some errors in the calculation of proximate digestibilities are inherent. Schneider and Ellenberger (1927) found that in dairy cows, the greatest error encountered when calculating digestion coefficients was due to daily irregularities in the amount of fecal excretion, especially at low levels of feed intake. Coprophagic behavior in several of the ponies prior to the collection periods, as well as wood chewing, may have also been a source of error. In this study chromic oxide was fed as an external indicator and total feces were not collected. However, Parkins et al. (1982) found that there was no difference in the apparent digestibility of complete diet cubes in mature Thoroughbred geldings when comparing the use of chromic oxide (95% recoverable) with complete fecal collection over a 5 d period. Similarly, Schurg (1985) found that there was no significant difference between total fecal collection and chromic oxide in determining digestion coefficients for mature ponies and horses consuming hay, hay-grain or whole corn plant diets.

Finally, digestibility may have been affected by pelleting the feed. Schneider and Flatt (1975) point out

TABLE 12. CALCIUM AND PHOSPHOROUS BALANCE IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
Calcium balance, g/d	-2.9 ^c	-4.4 ^{bc}	-5.6 ^{bc}	-6.1 ^b	-4.8 ^{bc}	-3.2 ^c	.96
Phosphorous balance, g/d	-3.8	-2.4	-4.2	-4.0	-2.8	-2.7	.73

^aStandard error of means.

^{b,c}Means in the same row with different superscripts differ significantly (P<.05).

that pelleting may reduce digestibility somewhat due to a faster rate of passage through the gastrointestinal tract, however, increased rate of passage may increase intake in ad libitum fed animals. Schneider and Flatt (1975) further point out that pelleting may decrease crude fiber digestibility. Haenlein et al. (1966) observed that horses consumed 21% more of a pelleted feed than loose hay, but that pelleting did reduce crude fiber digestibility. Hintz and Loy (1966), however, reported that pelleting increased ether extract digestibility, but did not affect the digestibility of the other proximate components in Thoroughbred and Quarter Horse fillies. They concluded that feeding the same levels of pelleted versus nonpelleted diets did not affect the apparent efficiency of feed utilization.

Nitrogen Utilization in Response to Conditioning

Nitrogen balance data is shown in table 13. There was no treatment difference in nitrogen intake, although there was a trend ($P=.07$) for the control group to be consuming less nitrogen (37.5 g/d) than the exercised group (43.5 g/d) in period 3. A period difference did exist, however. In period 3 both groups were consuming less nitrogen ($P<.05$) than in the first two periods, since they were fed to maintain zero body weight change. There was no treatment difference in fecal nitrogen excretion, however there was a period difference. Fecal nitrogen excretion in the third

TABLE 13. NITROGEN VALUES IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
N intake, g/d	55.4 ^c	57.5 ^c	37.5 ^b	54.9 ^c	57.7 ^c	43.5 ^b	2.1
Fecal N excretion, g/d	23.4 ^{bcd}	24.5 ^{cd}	17.8 ^b	23.0 ^{bcd}	26.0 ^d	19.0 ^{bc}	1.9
Urinary N excretion, g/d	20.6 ^c	21.3 ^c	14.0 ^b	22.0 ^c	19.8 ^c	16.8 ^b	.9
N balance, g/d	11.4 ^d	11.7 ^d	5.7 ^b	9.9 ^{cd}	11.9 ^d	7.7 ^{bc}	1.0
N absorbed, g/d	32.1 ^d	33.0 ^d	19.7 ^b	31.9 ^d	31.7 ^d	24.5 ^c	.9

^aStandard error of means.

^{b,c,d}Means in the same row with different superscripts differ significantly (P<.05).

period was similar to that observed in the first period, but less ($P < .05$) than that observed in the second period. It appears, then, that fecal nitrogen excretion is related to nitrogen intake. This agrees with work done in mature stock-type geldings by Freeman et al. (1981), Freeman et al. (1985b) and Hinkle et al. (1981), who observed a trend for increased fecal nitrogen excretion with increasing levels of nitrogen intake.

Urinary nitrogen excretion paralleled nitrogen intake. There was no treatment difference, but both groups excreted less urinary nitrogen ($P < .05$) in the third period than in the first two (14.0 g/d and 16.8 g/d for the control and exercised groups, respectively). This agrees with work done by Hinkle et al. (1981) and Freeman et al. (1985b) in conditioned geldings, and Slade et al. (1970) in Thoroughbred and Quarter Horse mares.

Nitrogen absorbed was less ($P < .05$) in the third period than in the first two for both groups and averaged 19.7 g/d for the control group and 24.5 g/d for the exercised group. In the third period, however, the exercised group absorbed more nitrogen ($P < .05$) than the control group. Freeman et al. (1985a) suggest that nitrogen metabolism may be affected by exercise in the horse, however, nitrogen absorption was related to intake, and the exercised group did tend to consume more nitrogen than the control group in the third period. Thus, the difference in intake may explain the

difference in nitrogen absorption.

No significant treatment differences were observed in nitrogen balance, however a period difference was again noted. In the control group, nitrogen balance in the third period (5.7 g/d) was lower ($P < .05$) than in the first two periods. In the exercised group, however, nitrogen balance in the third period (7.7 g/d) was less ($P < .05$) than in the second period, but was similar to values reported in the first period. Nitrogen balance, then, was higher at higher levels of nitrogen intake. This has been reported by Slade et al. (1970) and Harper and Vander Noot (1974) in sedentary horses, and Freeman et al. (1981) and Freeman et al. (1985b) in conditioned horses.

Nitrogen balance values obtained are within the range reported by Slade et al. (1970) in sedentary mares receiving diets containing 10 to 13.1% crude protein (100% DM basis). Values are also similar to those reported by Freeman et al. (1981) in mature sedentary geldings (11.1 g/d). While values are higher than those reported by Reitnour and Salsbury (1976) in ponies receiving diets containing 5.0% and 9.4% crude protein, they were similar to values reported when 9.7% and 10.0% crude protein diets were fed. Nitrogen balance values were much lower than those reported by Harvey et al. (1939) in draft geldings, and Hinkle et al. (1981) and Freeman et al. (1985a, 1985b) in stock-type geldings. Harvey et al. (1939) reported that there appeared to be no

obvious relationship between nitrogen retained and the severity of exercise. While Freeman et al. (1981) observed an increase in nitrogen balance with increasing workload, nitrogen intake was increased proportionally with workload, and as previously noted, nitrogen balance is affected by nitrogen intake (Hinkle et al., 1981; Freeman et al., 1985a, 1985b).

Correlations between nitrogen intake and other nitrogen values are shown in table 14. Nitrogen intake was highly correlated with fecal and urinary nitrogen excretion, nitrogen absorbed and nitrogen balance. This agrees with work done by Harper and Vandor Noot (1974), Slade et al. (1970), Reitnour and Treece (1971) and Reitnour and Salsbury (1976).

Nitrogen balance and nitrogen absorbed as a percentage of intake are shown in table 15. No period or treatment differences were observed for either parameter. Nitrogen balance as a percentage of intake averaged 19.0% for the control group and 18.6% for the exercised group over all periods, while nitrogen absorbed as a percentage of intake averaged 56.0% for the control group and 56.6% for the exercised group. Thus, when made relative to intake, there was no difference in the amount of nitrogen digested or retained.

Since there was no significant treatment difference in nitrogen balance, it appears that nitrogen balance is

TABLE 14. CORRELATIONS BETWEEN NITROGEN INTAKE AND OTHER NITROGEN VALUES

Variable	Correlation coefficient
Fecal nitrogen	.87*
Urinary nitrogen	.69*
Nitrogen balance	.69*
Nitrogen absorbed	.91*

*Significant correlation ($P < .001$).

TABLE 15. NITROGEN BALANCE AND NITROGEN ABSORPTION AS A PERCENTAGE OF INTAKE IN CONTROL AND ANAEROBICALLY EXERCISED PONIES

Variable	Treatment						SE ^a
	Control			Exercise			
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
N balance as % of intake	21.0	20.9	15.1	18.0	20.6	17.1	.02
N absorbed as % of intake	57.9	57.5	52.6	58.1	55.4	56.3	.02

^aStandard error of means.

affected more by nitrogen intake than by exercise. Harvey et al. (1939) found that exercise level (maintenance, light, medium or heavy work) did not significantly effect nitrogen balance in draft geldings. Freeman et al. (1981) observed an increase in nitrogen balance in geldings receiving an increasing daily workload and nitrogen intake. However, in a follow-up study, Freeman et al. (1985b) observed that nitrogen intake accounted for 64% of the variability in nitrogen balance in mature conditioned geldings, thus nitrogen balance was highly dependent on nitrogen intake.

Linear regression analysis was conducted to determine the effect of nitrogen intake on nitrogen balance. The relationship was analyzed by assigning nitrogen intake as the independent variable and nitrogen balance as the dependent variable. The coefficient of determination indicated that nitrogen intake accounted for 47% of the variability in nitrogen balance. The fitted line was determined to be: $Y = -1.979 + .229X$, where Y was nitrogen balance and X was nitrogen intake. The regression coefficient, .229, was significant at the .001 level and indicates that for each 1 g increase in nitrogen intake, nitrogen balance is increased by .229 g. This value, .229, is intermediate between that reported by Slade et al. (1970) in sedentary mares (.104 to .134) and Freeman et al. (1985b) in conditioned geldings (.412).

One problem encountered in nitrogen balance studies is

that nitrogen is lost through sweat (Calloway et al., 1971; Jenkinson et al., 1974; Snow et al., 1982; Kerr et al., 1983; Freeman et al., 1985a). Apparent nitrogen balance values, then, may be higher than true balance values. Sweating is quite variable, but Kerr et al. (1983) reported that the onset of sweating in Shetland and Welsh ponies was later than the onset in Thoroughbreds following epinephrine infusion and heat exposure. Consalazio et al. (1975) point out that nitrogen balance may be biased particularly when sweating is profuse. Thus, nitrogen lost in the sweat may have been a source of error in calculating nitrogen balance.

SUMMARY

No significant treatment differences were observed in any of the metabolic parameters measured during the treadmill tests. High heart rates and blood lactate concentrations indicate that the ponies were nearing maximal work. Since there were no treatment differences in any of the parameters measured, an exercise tolerance treadmill test may not be an appropriate measure of fitness in anaerobically trained ponies.

Muscle fiber composition may have been affected by anaerobic training, since the exercised group had a significantly lower percentage of slow oxidative fibers than

the control group.

Nitrogen intake was highly correlated with fecal and urinary nitrogen excretion, nitrogen absorbed and nitrogen balance. When nitrogen balance and nitrogen absorbed were examined as a percentage of intake, no period or treatment differences were observed. From these results it appears that nitrogen balance is affected more by nitrogen intake than by anaerobic exercise training. Since positive nitrogen balance values were observed in every period, it appears that ponies undergoing intense anaerobic exercise training may not have an increased need for dietary protein.

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APPENDICES

APPENDIX A. PRE AND POST-TRIAL BODY WEIGHTS IN CONTROL AND ANAEROBICALLY EXERCISED PONIES.

Pony number	Treatment	Pre-trial wt, kg	Post-trial wt, kg
1	E ^a	160.0	160.9
2	C ^b	170.9	179.5
3	E	156.8	160.0
4	C	187.7	191.4
5	E	199.5	205.0
6	C	130.0	128.6
7	E	134.1	136.8
8	C	145.5	144.5
avg		160.6	163.4
SE ^c		9.8	9.8

^aExercise.

^bControl.

^cStandard error of means.

APPENDIX B. AVERAGE WEIGHT PULLED EACH WEEK BY
ANAEROBICALLY EXERCISED PONIES

Pony Number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
	wt pulled, kg					
1	78.6	80.9	92.7	96.8	102.9	108.2
3	82.3	80.9	92.7	101.4	108.2	108.2
5	117.2	127.3	141.2	147.3	147.3	147.3
7	78.6	82.3	100.0	116.7	125.7	125.0
avg	89.2	92.9	106.7	115.6	121.0	122.2

Appendix C. Staining Procedure for Alkali-Stable Actomyosin
ATPase Activity

Solutions:¹

1. Fixative (5% formalin buffered at pH 7.6)

Formaldehyde solution (40%)	50 ml
Na cacodylate (MW 160).	31 g
CaCl ₂ (MW 147).	10 g
Sucrose (MW 342).	115 g

Bring to final volume of 1 liter with H₂O.

2. Rinse solution 18mM CaCl₂ in 100 mM tris (hydroxymethyl) aminomethane (Tris), pH 7.8

Tris (MW 121)	12.1 g
CaCl ₂ (.18 M)	100 ml
Distilled H ₂ O	900 ml

Adjust pH to 7.8 with HCl (1 to 6 N) using pH meter, and bring final volume to 1 liter with H₂O.

3. Alkaline preincubation (18 mM CaCl₂ in 100 mM buffer, pH 10.4)

Sigma No. 221 buffer ² (1.5 M)	3.35 ml
CaCl ₂ (.18 M)	10 ml
Distilled H ₂ O	30 ml

Adjust pH to 10.4 with KOH (1 to 10 N) using pH meter, and bring final volume to 50 ml with H₂O.

4. Incubation solution (2.7 mM ATP, 50 mM KCl, 18mM CaCl₂ in 100 mM buffer, pH 9.4)

Sigma No. 221 buffer (1.5 M).	3.35 ml
CaCl ₂ (.18 M)	5.00 ml
KCl (MW 75)185 g
ATP, Disodium ³ (MW 551.2)076 g
Distilled H ₂ O	40 ml

Adjust pH to 9.4 with 6 N HCl (using pH meter) and bring final volume to 50 ml with H₂O and filter into 60 C Coplin jars.

5. Wash solution (1% CaCl₂, w/v)

CaCl ₂ (MW 147).	10 g
Distilled H ₂ O	1000 ml

6. Cobalt chloride solution (2% w/v)

CoCl ₂ (MW 238).	1 g
Distilled H ₂ O	50 ml

Appendix C (Continued)

7. Alkaline washing solution (100 mM buffer, pH 9.4)
Sigma No. 221 buffer (1.5 M) 13.4 ml
Distilled H₂O 160 ml
Bring pH to 9.4 with HCl (1 to 6 N) using pH meter and adjust to final volume of 200 ml with H₂O.
- 8: Ammonium sulfide solution (1% v/v)
Ammonium sulfide (light)⁴5 ml
Distilled H₂O 50 ml

¹Solutions 1 and 2 are stable and, therefore, large quantities can be prepared and stored in the refrigerator. The other solutions are not stable and are prepared before use. (The 50 ml volume described is adequate to fill a Coplin staining jar.) The pH of buffer solutions in the alkaline range (Solutions 3, 4 and 7) is not stable because the solutions tend to absorb CO₂ from the atmosphere. These solutions must, therefore, be prepared immediately before use.

²Sigma No. 221 buffer is a trade name for a 1.5 M solution of 2-amino-2-methyl-1-propanol that is obtainable from the Sigma Chemical Co., 3500 DeKalb Street, St. Louis, MO 63118.

³Sigma Chemical Co., St. Louis, MO.

⁴This reagent deteriorates with age. It should be replaced if mottled, uneven staining of the tissue occurs.

Procedure:

1. Fix sections for 5 min in Solution 1.
2. Rinse slides in Solution 2 for 1 min with agitation and drain excess solution on blotting paper.
3. Preincubate in Solution 3 for 15 min (at this point make up Solution 4).
4. Rinse slides in Solution 2 (two changes, 1 min each) and drain excess solution.

Appendix C (Continued)

5. Incubate for 60 min in Solution 4 at 37 C. (The solution 4 is filtered into a staining jar that is prewarmed to 60 C. This rapidly warms the solution to about 37 C).
6. Wash in two 45 s changes of Solution 5 and drain excess solution.
7. Place in Solution 6 for 3 min.
8. Wash in one 30 s and two 60 s changes of Solution 7 and drain excess solution.
9. Place in solution 8 for 3 min.
10. Wash in running tap water for 3 to 5 min, dehydrate in graded ethanol, clear in toluene and mount in Permount.

Appendix D. Staining Procedure for Reduced
Diphosphopyridine Nucleotide
Tetrazolium Reductase (DPNH-TR)
Activity

Incubating Solution: Make up fresh

- | | |
|--|--------|
| 1. 0.2 M "tris" buffer* (pH 7.4) | 10 ml |
| 2. Nitro blue tetrazolium. | 2.5 mg |
| 3. Reduced diphosphopyridine nucleotide. . . | 8 mg |

Procedure:

1. Incubate sections in the above solution for 30 min at 36 C.
2. Take through ethanol-water mixtures of the following strengths in the order given:
(Approximately 5 min each.)

50/50
75/25
95/5
100/0

Rinse in toluene for 5 min.

Mount in Permount.

*Tris Buffer pH 7.4

- | | |
|---|-------|
| 0.2 M tris (hydroxymethyl) amino methane
2.423 g in 100 ml distilled H ₂ O) | 25 ml |
| 0.1 M hydrochloric acid | 42 ml |
| Distilled H ₂ O | 58 ml |

NITROGEN UTILIZATION AND METABOLIC RESPONSES OF PONIES
TO INTENSE ANAEROBIC EXERCISE

by

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Eight mature pony geldings with an average weight of 161 kg were randomly assigned to either a control (no exercise) or a treatment group to examine the effects of intense anaerobic exercise on metabolic responses to exercise tolerance tests on the treadmill, muscle fiber type and nitrogen utilization. Treated ponies were worked by pulling a weighted sled for 10 min/d, 5 d/wk for 6 wk. Total weight pulled ranged from 79 to 147 kg and was adjusted daily if necessary to maintain heart rates of 170 to 190 beats/min throughout each 10 min work session. All ponies were subjected to 30 min exercise tolerance treadmill tests on days 0, 21 and 42 of conditioning. There were no treatment differences in the metabolic parameters measured (heart rate, respiration rate, blood lactate concentration and rectal temperature). Heart rate at 30 min of the test averaged 206.8 beats/min and 208.5 beats/min for the control and exercised groups, respectively, over all test days. Blood lactate concentration immediately following the test averaged 14.4 mmol/liter for the control group and 13.3 mmol/liter for the exercised group over all test days. High heart rate and blood lactate values indicated that ponies were nearing maximal work. Since no treatment differences were observed in any of the parameters measured, an exercise tolerance test on the treadmill may not be an appropriate method for evaluating fitness in anaerobically trained ponies. Muscle biopsies were taken from the left biceps femoris of all ponies at the end of the 6 wk training

period. Muscle fiber composition averaged 27.1% FG, 53.4% FOG, and 19.5% SO for the control group and 34.9% FG, 56.6% FOG, and 8.5% SO for the exercised group. There was no treatment difference in percent FG and percent FOG, however, percent SO was significantly lower in the exercised group. Six day nitrogen balance trials were conducted on both groups prior to conditioning and on days 1 to 6 and 37 to 42 of conditioning. There were no significant treatment differences in fecal and urinary nitrogen excretion or nitrogen balance. In the third period nitrogen absorbed was higher in the exercised group (24.5 g/day) than in the control group (19.7 g/day), however nitrogen intake tended to be higher in the exercised group. When analyzed relative to intake, though, there was no period or treatment difference in nitrogen balance or nitrogen absorbed. Nitrogen intake was highly correlated with fecal and urinary nitrogen excretion, nitrogen balance and nitrogen absorbed. It appears, then, that nitrogen balance is affected more by nitrogen intake than by anaerobic exercise training. Since positive nitrogen balance values were maintained throughout the trial, ponies undergoing intense anaerobic exercise training may not have an increased need for dietary protein.