PHYSIOLOGICAL EFFECTS OF DIET AND EXERCISE IN THE EQUINE.

by

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(ABSTRACT)

Two experiments were conducted to investigate the effect of conditioning on the ability of the equine to digest and utilise nutrients and to determine the effect of dietary fat as an energy source on the physiological parameters associated with fitness using a standard exercise test. Conditioning horses increased apparent digestibilities of crude protein (CP) ($P<.05$), dry matter(DM) and acid detergent fiber (ADF) ($P<.1$). Conditioning also tended to increase the apparent digestibility of neutral detergent fiber (NDF), cellulose, cell contents, and energy. Heart rates and blood lactate levels indicated that the conditioned horses were fitter than their unconditioned controls. In the second experiment horses were fed isocaloric diets, one containing added fat and the other a standard hay/corn diet. Adding fat while maintaining equal available energy concentration depressed apparent digestibility of dry matter (56.7 vs 67.3 % $P<.05$), cell contents (75.6 vs 82 %, $P<.05$), energy (61.2 vs 71.8 %, $P<.05$) and NDF (29.2 vs 51.3 %, $P<.05$), in unconditioned horses. There was a trend towards decreased apparent digestibility of CP and ADF. Addition of fat increased apparent digestibility of ether extract (89.2 vs 65.6 %, $P<.05$). Conditioning increased apparent digestibility of CP (64.8 vs 73.7 %, $P<.05$) and energy (61.2 vs 65.6 %, $P<.05$) and tended to

increase apparent digestibility of DM (56.7 vs 60.8 %) and ADF (26.8 vs 17.8 %) for horses fed a fat supplemented diet. Conditioning did not cause a change in apparent digestibility of ADF, CP, and DM in horses fed the control diet, or apparent digestibilities of NDF, ether extract, cell contents, or energy for either diet. There were no differences in physiological parameters used for assesing fitness (heart rate, blood lactate and respiration rate), between horses fed a diet containing 14% added fat and no added fat. There was no difference in body temperature, blood glucose levels, blood urea-N (BUN) or creatine phosphokinase (CPK) between horses fed the two diets.

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Introduction

Great changes in the popularity of horses have occurred this century. The number of horses in regular use as work animals declined until the end of World War II. However, presently there are more horses than ever before in many varied roles which provide sport entertainment and employment to millions of people. The American Horse Council (AHC, 1987) estimates that there are 5.25 million horses in the United States, with the industry providing $15 billion annually to the U.S economy. Many of these horses are used for strenous sports such as racing, horse trials, show jumping, endurance riding, cutting, roping and driving. The rise in value and economic importance of these animals have been enormous; star performers in these various activities change hands for thousands, and in some cases millions of dollars. Prize money and purses have risen astronomically and there is now a huge international market in sport and performance horses. Standards of performance have risen, and to win a major international competition today requires a very different standard of fitness, training and athletic ability, compared to the immediate post war era. There has also been a massive rise in interest in the horse for leisure pursuits, and a whole industry has been built up around the use of the equine as an athelete.

This increased interest in the equine as an athelete has stimulated research into their nutritional needs, including limited research into the needs of sport horses. Although research on the nutrition of the horse has intensified during recent years, little has been done on the

nutritional requirements for strenous work, nor has much been done on the effect of fitness training and exercise on the efficiency of digestion.

With the increased levels of performance demanded from equines in the more demanding equestrian sports, horsemen have had to find ways of increasing the energy availible to the animal. Traditionally, this has been done by increasing the level of readily-digestibile carbohydrate in the diet, such as feeding a higher percentage of grains. This is not without price, since increasing percentages of grain or concentrates in the equine diet can lead to many problems and complications such as founder (laminitis), colic and other digestive upsets. Furthermore there is some evidence that high levels of carbohydrate feeding may predispose an animal to exertional myopathy or rhabdomyolysis (Lindholm 1982; McLean 1966), but this is not yet proven.

Animals fed a high level of grain and in very hard work may suffer from a condition known as overtraining or "going over the top", the symptom of which is when the animal goes off feed and loses interest in eating. This can have devastating effects on a carefully planned and prepared training or competition schedule.

Fat contains 2.25 times as much energy per gram as carbohydrate, a fact which has stimulated research into use of fat as an energy source for livestock. A modern trend in human nutrition has been against consumption of fat and thus some of the traditional fats used in human food have fallen into disrepute and increased amounts of these foodstuffs have begun to appear on the market aimed at the livestock industry.

Experiments were conducted to study the influence of conditioning equines on digestion, and to determine the value of added fat on physiological parameters associated with fitness.

Literature Review

Anatomy and Physiology of Muscle

Structure. All movement in vertebrates is brought about by muscular contractions. Exercise training brings about changes in the structure and metabolism of muscles (Goll et al., 1984). The muscle cell is an elongated multinucleated cell, generally referred to as a fiber. Over 90% of muscle consists of muscle fibers. The remaining 10% consists of nerves, blood vessels and connective tissue which separates individual muscle fibers (endomysium), and muscle bundles (perimysium). The connective tissue merges with tendons, which anchor the muscles to the bones.

The diameter of muscle fibers varies within and between muscles, the size being affected by growth and activity (Snow, 1983; Essen et al., 1980). It is generally accepted that increased muscle mass after birth is brought about by an increase in size of individual fibers (hypertrophy) rather than increased numbers (hyperplasia).

Within the muscle fiber, hundreds of myofibrils, the contractile elements, are aligned parallel to each other (Goll et al., 1984). A myofibril consists of repeating units, the sarcomere, each of which is capable of generating tension. When all the sarcomeres of a fibril contract together the fibril exerts a pulling force. Within each sarcomere, strands of the contractile proteins tropomyosin and troponin lie around

actin filaments. The myosin strands vary slightly in composition between different fiber types, which result in different responses between fibers (Snow, 1983). Myosin consists of a long "tail" (light meromyosin) with a heavier "head" (heavy meromysin) at regular intervals. The heads form the cross bridges which contain the myosin ATPase which is responsible for releasing the energy needed for contraction.

Contraction: The precise mechanism of contraction has yet to be fully elucidated. During contraction the sarcomeres shorten, resulting from the actin filaments sliding between myosin filaments as a result of interaction of the myosin crossbridges with actin (Goll et al., 1984). This is prevented in the resting state by the inhibitory action of troponin. However, release of Ca ions from the sarcoplasmic reticulum deactivates the troponin, which allows the myosin to move. The cross-bridges are now able to attach to actin molecules and splitting of ATP provides the necessary energy for conformational changes in the head, resulting in the actin filament sliding along the myosin filaments. Each sarcomere contains thousands of cross-bridges and the extent of contraction will depend on the number of cross-bridges activiated.

Relaxation occurs when new ATP is taken up by the myosin heads which then disassociate from the actin. The Ca which is released from troponin, is actively transported back into the sarcoplasmic reticulum (also ATP requiring). The tropomysin complex is once more inhibitory. The ATP required for myofibrillar contraction is supplied from one of two sources, either from glycolysis in the cytoplasm of the muscle cells or from the mitochondria which is where oxidative phosphorylation takes place (Goll et al., 1984)

Skeletal Muscle Fiber Types. Early sources differentiated between muscles based on color (red or white), which was a reflection of their myoglobin content (Ranvier, 1874; Needham, 1974). Red muscle is high-oxidative muscle (high myoglobin) and white muscle is a low oxidative muscle (low myoglobin). More recently, muscle fiber types were differentiated on the basis of contractile properties. There are two distinct types; slow twitch or type I fibers, which have low myosin ATPase activity at a pH of 9.4, and a relatively slow contraction and relaxation time. (Barany 1967; Burke, et al., 1973; Garrett, et al., 1978). Fast twitch or type II have a relatively faster contraction and relaxation time and a high myosin ATPase activity at pH 9.4. Brooke and Kaiser (1970) further subdivided the type II fibers into type IIA and IIB, according to the lability of their myosin ATPase at acidic and high alkaline pHs. They also identified an immature transitional type IIC fiber which was found in young animals during development and in mature animals as a transitional fiber. This has been supported by Snow et al (1981), using a histochemical technique. The differences in myosin ATPase activity between fibers has been explained as resulting from minor variations in the composition of myosin in these fibers, referred to as isomyocins (Weeds, 1980; Hoh et al., 1980; Howald, 1982; Pierobon-Bormoli, et al., 1981). Essen et al. (1980) used this classification system in a study of fiber types in the Standardbred.

Other classification systems are based on the metabolic properties of fibers, of which one of the simplest is the slow twitch (ST), fast twitch high oxidative (FTH), and fast twitch low oxidative (FT) system, which corresponds with the slow oxidative (SO), fast oxidative/glycolytic

(FOG) and fast glycolytic (FG) system used previously. Lindholm and Piehl (1974) and Snow and Guy (1977) used the ST, FTH, FT system in their studies on equine muscle physiology. This system is based on the measurement of two enzymes, one representative of oxidative capacity, either succinic dehydrogenase, or nicotinamide adenine dinucleotide diaphorase, and the other of glycolytic capacity, usually but not always, phosphorylase or alpha-glycerol phosphate dehydrogenase.

In young horses a fairly clear distinction can be made on the basis of oxidative capacity, but as training occurs subdivision becomes more difficult as there is a general increase in oxidative ability, This is one reason many workers have now resorted to using a system based on myosin ATPase activity (Essen, et al., 1980; Snow, 1983).

Fiber Type Populations within Muscle. In the horse and man most muscles consist of a mosaic of the three major fiber types, which some workers (Johnson, et al., 1973; Edgerton, et al., 1975 and Snow, 1983) claim are spread uniformly thoughout the muscle. This is in contrast to laboratory animals and dogs in which the distribution of fiber types is not uniform (Snow, 1983). However, there is some contradiction, as Gunn (1978) and Raub et al. (1983) have reported a significant difference in the distribution of fiber types between deep and superficial regions of several muscles of the horse.

The different proportions of fiber types within the muscles reflect the different functional requirements of the various muscles (Lindholm and Piehl, 1974; Gunn, 1978; Snow and Guy, 1980; Snow, 1983).

Even within one muscle, marked variations in composition between mature individuals have been described in man and horse. In man the most

frequently studied muscle is the thigh muscle, the vastus lateralis, which has been found to have slow twitch fibers, varying from 10 to 100% of all fibers within that muscle, between persons, with a population mean of 50% (Saltin et al., 1977). Elite marathon runners have a higher proportion of slow-twitch muscle fibers, while elite sprint runners have a low proportion of slow-twitch fibers (Saltin et al., 1977; Gollnick, 1972).

Snow and Guy (1980) studied the proportion of fiber types in the limb muscles of a number of equine breeds. They found a relationship between performance ability and the proportion of fiber types in the middle gluteal muscle. Thoroughbreds and Quarter Horses have a higher proportion of type IIA (FT) and IIB (FTH) fibers, while draught horses, donkeys and Arabians had the highest proportion of type I (ST) fibers. This is in agreement with Barlow et al. (1985), who also found a correlation between percentage of type IIA and IIB fibers and subsequent racing record in 14 Thoroughbred yearlings. Komi et al. (1977) suggested that the factors controlling the proportion of slow and fast twitch fibers are genetically determined. However Howald (1982) has suggested some instances where enviromental factors may alter the proportions.

Pette (1980) showed that by drastic means such as cross- innervation and chronically implanted electrodes fiber types can be completely transformed from type I to type II and vice-versa. The change in the molecular structure of myosin occurs following alterations in metabolic properties and capillarity. Such extreme changes are not thought generally to occur with normal training methods. However, Jansson et al. (1978) and Howald (1982) have suggested that this may take place via the transitional IIC fibers when long endurance training is undertaken. In

the horse, Guy and Snow (1977a) reported a slight, but significant increase in type I fibers in six limb muscles studied during training programm consisting of aerobic and anaerobic training. Snow (1982) reported similar findings in a study of 70 horses biopsied before training and again 8 mo later. A higher ratio of type 11A and 11B fibers has been shown to occur in endurance trained humans (Saltin et al., 1977; Gollnick et al., 1973), and in Standardbred horses (Essen et al., 1980).

Snow and Guy (1981) compared fiber composition of the middle gluteal muscle in Thoroughbred horses of various ages from birth upwards and in various stages of training. They found that although there was a steady decrease in the proportion of type IIB with age from foals to the oldest animals in training, a training effect separate from age could not be shown.

Fiber Area. In addition to the significance of the proportion of fast and slow twitch fibers the cross sectional area of the individual fibers is also important, as this influences force output. In man it has been shown that the greater the cross sectional area of fast twitch fibers the greater the force output (Gollnick, 1982). Where strength-only exercises are involved, such as weight lifting, hypertrophy of fibers with increased strength have been reported (Gollnick et al., 1972; Costill et al., 1979). It has been suggested that increased strength may occur in the absence of hypertrophy due to improved efficency of motor recruitment (Gollnick, 1982). Gollnick et al. (1973) reported an increase in the area of type I fibers and a small decrease in type II area. Lindholm and Piehl (1974) reported an increase in cross sectional area of all fiber types with age, and for training in Standardbreds. Nimmo et al. (1982) reported

an increase in the percentage of ST fibers after endurance training and a decrease in FT fibers. It seems likely that changes seen reflect the extent and duration of training.

Capillary Number. In man there have been several studies on oxygen uptake and capillary density. It has been reported that there are a similar number around all fiber types (four to five capillaries per fiber) (Saltin et al., 1977), and conversely that there are fewer around type IIB than around IIA fibers (Anderson, 1975; Ingier, 1979).

In the horse there are only three reports on actual measurement of capillary density around specific fiber types. Nimmo et al. (1982) found a similar number of capillaries around both high and low-oxidative fibers. When expressed in terms of capillaries per fiber area, the low-oxidative (type IIB) fibers have the poorest supply. This finding was in agreement with Henkel (1982, 1983), who found that the highest diffusional capabilities (small fibers surrounded by many capillaries) were displayed by the type I fibers and the lowest by the type II fibers. This is in keeping with their respective capabilities for aerobic metabolism.

Endurance training has been reported to cause an increase in capilliary numbers (Saltin et al., 1977; Anderson and Henrikson, 1977). Anerobic training was found to decrease the capillarization of IIA fibers (Daub et al., 1982).

Muscle Biochemistry

Numerous studies have been carried out in man and laboratory animals to investigate alterations in key enzymes within the different metabolic pathways important in the generation of ATP. There is considerable var-

iation in responses reported by various workers, although direct comparison between results obtained is difficult due to the varying types, intensities and durations of the exercise and training programs used.

Endurance Training. In general, endurance training appears to increase the overall activity and concentration of enzymes representative of oxidative phosphorylation, although the relative capacities may vary (Gollnick, 1982; Snow, 1984). The enzymes studied include succinate dehydrogenase, citrate synthetase, cytochrome C and malate dehydrogenase. These changes parallel an increased mitochondrial volume. Many workers also report an increase in 3-hydroxyacyl CoA dehydrogenase activity (Carlson 1978; Straub et al., 1983) This is an enzyme involved in fatty acid degradation. The significance of these changes in oxidative capacity, has been discussed in a number of reviews, (Howald, 1982; Gollnick and Saltin, 1982; Snow, 1983). The importance of the increased oxidation of fatty acids with reference to decreased glycogen utilization and lactate production (glycogen spareing) is discussed in the review by Gollnick and Saltin (1982).

There have been no reports of an increase in the glycolytic enzymes, lactate dehydrogenase, phosphorylase, phosphofructokinase, glyceraldehyde phosphate dehydeogenase, hexokinase, triosephosphate dehydrogenase, and glycogen synthetase with endurance training. Some workers (Snow, 1977, 1983), have reported a decrease in total lactate dehydrogenase activity, as well as the formation of the cardiac isoenzymes of lactate dehydrogenase. Essen- Gustavsson et al. (1983) reported a decrease in lactate dehydrogenase activity in young Standardbred colts

with age, which was not different from young Standardbreds of the same age which had undergone a training program.

High-Intensity Training. There has been much less work on high-intensity sprint type (anaerobic) training programs, which result in predominately anaerobic work. Roberts et al. (1982) used a high intensity program in human runners and found increases in lactate dehydrogenase, phosphorylase, phosphofructokinase and glyceraldehyde phosphate dehydrogenase. In an earlier study Sjodin et al. (1976) used a much less intense work load and found no significant change in any of these enzymes nor in the activity of the oxidative enzyme, succinate dehydrogenase. Fourrier et al. (1982) studied the effect of sprint running in adolescent boys and found an increase in phosphofructokinase activity, but no changes in any of the other glycolytic enzymes or succinate dehydrogenase. The effects of varying intensities and duration of daily exercise on cytochrome C in rats have been studied by Dudley et al. (1982). They found that changes in activity and concentration were dependant on both duration and intensity of work. In the type IIB (fast twitch, low oxidative) fibers, increases in cytochrome C concentration only occurred after exercise above a certain intensity. This may be because of the patterns of fiber recruitment. No changes can be expected to occur if the fiber is not recruited, and the IIB fibers will not be recruited unless the exercise is near maximal intensity or until other fibers are glycogen depleted (Snow et al., 1982b).

Combined Training. Guy and Snow (1979) studied the effects of a combined anaerobic, aerobic training program lasting 10 to 15 wk on the

activities of various enzymes in six limb muscles in the horse. They found increases in the activity of all seven enzymes measured (alanine amino transferase, aspartate amino transferase, aldolase, creatinkinase, citrate synthetase, 3-hydroxyacyl CoA dehydrogenase and lactate dehydrogenase). All except aldolase showed the highest activity in the gluteus maximus. In a further study of the middle gluteal and some tendinosus muscles they found an increase in activity of 3-hydroxyacyl CoA dehydrogenase, but glycogen synthetase and hexokinase remained unaltered.

Snow et al. (1984) studied the effect of race training on muscle enzyme activities in Thoroughbreds. They found a small increase in hexokinase, a decrease in fructose 1-b-diphosphatase, no change in phosphofructokinase and creatinphosphokinase activity, and an increase in citrate synthase. These authors concluded that a programme of mixed aerobic and anaerobic work results in adaptation of both the glycolylic and oxidative pathways. Nimmo et al. (1982), in a cross-over study of anabolic steroids and exercise in Thoroughbreds, using a less strenous training program found increases in 3-hydroxyacyl CoA dehydrogenase, and citrate synthase, but these were less marked than in the previous study. No alteration occurred in the activity of the glycolytic enzymes, phosphofructokinase and lactate dehydrogenase. These different results may reflect the lower intensity and shorter duration of the training program.

Lindholm and Piehl (1974) and Essen et al. (1980) compared young Standardbreds before training with older animals in training. Lindholm and Piehl (1974) found an increase in succinate dehydrogenase with age

and training while phosphofructokinase first increased and then decreased. Essen et al. (1980) found an increase in oxidative capacity and 3-hydroxyacyl CoA dehydrogenase activity, but a decrease in glycolytic capacity. In a later study Essen-Gustavsson et al. (1983) studied the effect of growth and conditioning on very young Standardbreds. They biopsied foals 6 to 8 mo of age, divided them into two groups, exercised (T group), and turned to pasture (C group). The horses were biopsied again at 11 to 12 mo and at 18 mo. They found a decrease in activity of lactate dehydrogenase and triose phosphate dehydrogenase with increasing age, but no difference due to training. Activity of 3-hydroxyacyl CoA dehydrogenase was not affected by age or training. Training significantly increased the activity of citrate synthetase. That this was a training effect is supported by the findings of Kline and Bechtel (1987), who found a slight decrease in citrate synthetase in muscle of Quarter Horses and Standardbreds from birth to 1 yr.

Energy Metabolism.

Effect of Exercise. One of the effects of exercise training is enhancement of Adenosine Triphosphate (ATP) producing systems in the muscle cells, particularly the activities and capacities of enzymes involved in oxidative metabolism. An increased capacity for oxidative metabolism would mean that the energy requirements of the cells could be met from oxidative phosphoylation and the Krebs cycle which takes place in the

mitochondria. The primary substrate used for the Krebs cycle is acetyl Coenzyme A (Acetyl CoA). The two carbon unit (acetyl) can be derived from either glycolysis via pyruvate, or from the systematic degredation of fatty acid chains (a-oxidation) involving a chain of enzymes which include 3- hydroxyacyl CoA dehydrogenase. It has beeen shown that as exercise of low to moderate intensity increases in duration, the contribution of energy from fat increases (Snow, 1983). Hurley et al. (1986) showed that for exercise of the same intensity and duration in humans the proportion of energy derived from fat increased from 35% to 57% with training. A similar effect has been shown in rats (White and Block, 1985; Gleeson and Waring, 1987), humans (Moesch and Howald, 1975; Howald, 1982; Gollnick, 1985) and horses (Snow, 1977, 1979, 1982, 1983; Hoeppler et al., 1982; Essen-Gustavsson et al., 1983).

Mitochondria. Straub et al. (1975) showed an increase in mitochondrial volume with training in racehorses and enhanced activity of the Krebs cycle enzymes. In a later study Straub et al. (1983) found that with mixed aerobic and anaerobic training there was a large increase in the volume density of mitochondria and in the activities of glyceraldehyde 3-phosphate dehydrogenase and malate dehydrogenase, but not in 3-hydroxyacyl CoA dehydrogenase nor in muscle triglyceride levels or intracellular fat levels. They concluded that fat metabolism in the horse is of less significance than carbohydrate metabolism. However the training program used was only 4 wk. In those papers which report increases in 3-hydroxyacyl CoA dehydrogenase activity, training periods

were usually much longer (Snow and Guy, 1979; Essen et al., 1980; Nimmo et al., 1982).

Fatty Acid Metabolism. Carlson et al. (1964), found that turnover of free fatty acid (FFA) in equines was increased by exercise, partially by increasing the uptake from plasma and by increasing release from adipocytes, hence increasing plasma levels of FFA and glycerol. Administration of epinephrine to exercising horses enhanced the FFA turnover due to exercise and also increased plasma glycerol and lactate levels. In contrast Miller et al. (1963) showed a decrease of plasma FFA in dogs in response to exercise, which was due not only to increased uptake from plasma but also a reduced output by adipocytes. The reduction in lipolysis reported may have been due to the high plasma glucose and lactate levels, which have been reported to have an inhibitory effect on lipolytic activity (Havel et al., 1963; Carlson and Oro, 1963).

In man, Friedberg et al. (1963) showed that exercise increased plasma FFA turnover, but that there was a delay in response of FFA output in both the onset of exercise and after the exercise stopped. The rise in FFA could be due to the action of epinephrine, which was shown by Dole (1955) to have the most marked effect on FFA release. Insulin and glucose tending to have a depressive effect on plasma FFA levels.

The uptake of FFA from plasma by cells has been shown to be dependant on on the level on circulating FFA particularly the low density lipoprotein (LDL), and very low density lipoprotein (VLDL) fractions, and is enhanced by the the presence of oxygen and by epinephrine and norepinephrine (Evans and Mueller. 1962). Glucose has an inhibitory ef-

fect on uptake of FFA from plasma by cells, as does insulin (Heindel et al., 1974).

Endurance exercise augments plasma triglyceride clearance, causing a rise in high density lipoprotein (HDL), cholesterol and increasing the activity of lipoprotein lipase (Askew, 1984; Sady et al., 1986).

In the horse it has been shown that non-esterified fatty acids are important sources of energy during prolonged exercise (Hintz, 1983). Rose and Sampson (1982) showed a marked increase in plasma non-esterified fatty acid values with exercise.

It has been suggested by several authors (Dybal et al., 1980; Lucke and Hall, 1980; Naylor et al., 1980; Rose and Sampson, 1982) that the ketone pathway is not important in the horse. This may be due to a high capacity for liver glycogenolysis (Rose and Sampson, 1982). It must also be remembered that the horse being a cecal fermenter, obtains a good supply of volatile fatty acids (VFA) from the cecum and colon, of which propionate can be used for gluconeogenesis (Hintz, 1983).

Plasma Glucose Levels. The effect of exercise on plasma glucose levels appears to depend on several factors, such as speed and duration of exercise as well as state of training. In endurance type events plasma glucose levels appear to rise until 40 km (Lucke and Hall, 1980; Rose et al., 1980; Snow et al., 1982), but when measured at the 80 km there is a reduction in plasma glucose (Rose and Sampson, 1982; Snow et al., 1982). It has been suggested (Snow et al., 1982) that the increased glucose is due to mobilization of glucose from the liver. This could be because of the increased glucagon and decreased insulin concentrations, normally

seen with exercise (Felig and Wahren, 1975). After 4 h of steady exercise, up to 75% of liver glycogen is used and so gluconeogenesis is reduced. At the same time the muscles are absorbing more glucose from the peripheral circulation as the glycogen stores are depleted, so blood glucose levels drop. Thus, animals with a greater ability to use FFA can spare liver and muscle glycogen and delay onset of fatigue.

Muscle Glycogen and Lactate. In nearly all the papers where it is reported, training causes an increase in resting muscle glycogen concentrations, exertion causes a decrease, the degree of which is related to the intensity and duration of the exertion, but the overall effect of training is to increase muscle glycogen stores in all types of fibers (Gollnick, 1982; Snow, 1984; Essen et al., 1984).

Training also tends to decrease muscle and plasma lactate levels for a given level of exercise (Persson, 1983; Snow, 1984). The plasma level reflects a balance, and lower concentrations may be due to an increased uptake of lactate by liver and other muscles and/or reduced muscle production. Level of lactate production is higher in high intensity exercise of short duration, relative to exercise of longer duration and lower intensity (Persson, 1983), which may be a reflection of the pattern of fiber recruitment. Higher levels of exercise tends to result in increased recruitment of type IIB fibers which have a very low oxidative capacity, therefore producing a relatively high amount of lactate (Snow, 1983). This effect of increased muscle storage of glycogen with work and rapid release of lactate is particularly marked in the horse, compared to humans (Nimmo et al., 1982; Snow, 1983). In man lactate has been

suggested as the major contributor in promoting muscle fatigue (Snow, 1983), but remains to be investigated in the horse.

The horse tends to accumulate higher levels of glycogen in trained resting muscle than man (Snow, 1983), and tends to tolerate higher level of lactate (Snow, 1983). Snow et al.(1985) investigated lactate production and glycogen utilization in maximally exercised Thoroughbreds and found that they had muscle lactate concentrations up to 40 mg/kg dry muscle and blood levels in excess of 200 mmol/liter for periods of more than 30 min without ill effect. It has been suggested that high muscle lactate may be one of the causes of a condition called rhabdomyolysis or azoturia (Mclean, 1966; Lindholm et al., 1974), but this is not proven. Gollnick et al. (1985), working with humans, found that lactate production in exercising muscle is related to the amount of glycogen stored. In their study in which both legs were exercised, but one leg had been previously glycogen depleted, they found that although both legs had the same power output, there was a higher lactate output by the leg with the high glycogen.

Glycogen Loading. This refers to the over-compensation of muscle glycogen following severe depletion, and is practiced by human athletes, in order to delay fatigue in endurance type competitions. While it has been shown that it is possible to increase the muscle glycogen stores in equine by dietary manipulation (Kline and Albert, 1982; Topliffe et al 1984), no advantages in terms of exercise performance was shown (Topliffe et al., 1985).

If high levels of glycogen result in higher levels of lactate (Gollnick et al., 1985), and lactate accumulation is implicated in onset

of fatigue (Snow, 1983; Sutton, et al 1981), and possibly in rhabdomyolysis, then the benefit of this practice in equines would seem doubtful, particularly in view of the naturally high muscle glycogen content in the horse (Snow, 1983).

Exercise Testing. Plasma accumulation of lactate, which reflects the balance between output by working muscle and uptake by liver and non working muscle (Brooks and Kaiser, 1977), is exponentially related to both exercise heartrate and work load, expressed as velocity (Persson 1983). Thus, an incremental treadmill test allows the calculation of the blood lactate at the workload causing heartrate to equal 200 beats/min which is called the LA_{200}. Appropriate fitness training has the effect of lowering the blood plasma levels for a given exercise load (Snow, 1983; Persson, 1983). So well documented is this effect that as long as the speed and type of exercise are clearly defined, some workers claim that the plasma lactic acid (LA), the plasma lactic acid at the speed of 500 m/min (LA_{500}) and the velocity at which there is a blood lactate concentration of 4 mmol lactic acid/liter (V_4), could be used for further evaluation of aerobic capacity in endurance horses (Straub et al., 1983; Krusic et al., 1987).

The blood lactate level of 4 mmol/liter is considered to be the approximate level of exercise induced onset of blood lactate accumulation (OBLA) (Brooks and Kaiser, 1977; Persson, 1983). It is sometimes referred to as the anaerobic threshold (Kindermann et al., 1979; Sjodin and Jacobs, 1981a,b; Kumagi et al., 1982; Persson, 1983), and is used in exercise testing as a measure of fitness.

The significance of the LA_{200} is not clearly defined but it may be an indicator of the degree of metabolic intensity at speed in m/min at which the heartrate equals 200 beats/min (V_{200}), In man, trained individuals have slower glycogen utilization and lower muscle and lactate levels during submaximal exercise than untrained individuals, possibly due to inhibition of anaerobic glycolysis by increased oxidation of fats (Essen, 1977). Therefore, LA_{200} may be an expression for the degree of adaptation to training at least down to a minimum of 2 mmol/liter (Persson et al., 1983). The variation of heartrate response to exercise is very large, and as the LA_{200} is dependant on heartrate, it probably provides little information beyond the exercise heartrate. If this variable is related to performance capacity and/or fitness, this should be reflected in significant correlations with the other variables measured. The lower production of and less efflux of lactate from muscle cells may be due to the effect of catecholamines, epinephrine and norepinephrine. These stimulate glycogenolysis, which increases pyruvate levels thus increasing lactate production.

Exercise training may reduce the level of response to epinephrine or reduce the amount of sympathetic activity, and thus lower the epinephrine produced (Gollnick et al 1985). The slower rate of clearance of lactate from muscle in horses may be due to a higher proportion of type IIB fibers and their relativly poorer circulation (Snow et al., 1985). Hermansen and Vaage (1976) showed that in man only 10% of the muscle lactate was released during recovery from anaerobic exercise; the balance was reconverted back into glycogen and stored in the muscle. These workers found that if the muscle cells were partially glycogen depleted

and then allowed to recover, glycogen synthesis was four to eight times faster than the rate of glycogen synthesis in humans who were fed high-carbohydrate diets. They found there was no large increase in uptake of circulating glucose to account for the increases in glycogen, and concluded that when a muscle cell is allowed to recover with ample O_2, lacatate can be reconverted back to glycogen. This has not been shown to happen in the horse.

Cardiovascular Response to Exercise

Persson (1983) described a standardised set of exercise tolerance tests designed to establish normal values for response to exercise stress in equines. The effect of exercise on heart rate has been extensively studied and is the subject of several excellent reviews (Englehart, 1977; Fregin, 1983). Heart rate has been measured in exercising horses by use of a heart rate meter with surface adhering electrodes attached at the sternum and withers and held in place by saddle and girth (Fregin, 1983).

Average resting heart rate in horses is 35 to 40 beats/min, depending on age and degree of fitness, and can rise to 240 beats/min at maximal or near maximal workloads in galloping horses (Asheim et al., 1970; Ehrlein et al., 1970a,b), and is similar in swimming horses (Asheim et al., 1970; Fregin and Nicholl, 1977). Heart rate is highly correlated with work effort in galloping horses (Ehrlein et al., 1970a, b; Lindholm and Saltin, 1974). The heart rate/velocity relationship in horses running up a slope is linear up to 480 m/min, after which there appears to be a tendency for the rate to level off. Cardiac output in the horse increases

five to six fold with exercise to an estimated maximum of 240 liters/min (Fregin, 1983).

Respiratory Response to Exercise

Derksen and Robinson (1980) and Derksen et al. (1982) reported that horses breathe around their relaxation volume at rest, suggesting that respiratory muscular activity occurs during both inspiration and expiration. Presumably the abdominal, intercostal and possibly the strap muscles of the neck are involved. Attenburrow (1971,1978,1982) and Hornicke et al. (1974) reported that breathing frequency is a function of stride frequency in galloping horses at higher speeds. Extension of the forelegs and trunk could presumably aid inspiration and flexion could aid expiration. In this respect it would be efficient if breathing frequency was at a rate dictated by stride frequency. Some endurance horses, however, show breathing frequencies higher than their stride frequency during or just after periods of very heavy work. This is possible if the animal is trotting rather than cantering and indicates that at times breathing frequency as controlled by stride frequency might not meet the O_2 requirement. Also, an increased body temperature from exercising or from high environmental heat and humidity will also cause a horse to breathe faster (Gillespie and Pascoe, 1983).

Hornicke et al. (1983) developed a telemetric system for measuring tidal volume, O_2 consumption and O_2 pulse of riding horses at walk, trot

and gallop in a field. They found that O_2 consumption and pulmonary ventilation were almost linearly related to riding velocity. The cost of carrying 1 kg body weight over 1 m was .2 ml O_2 and was not significantly different for walk, trot and gallop. The amount of air ventilated to obtain 1 liter of O_2 declined from 40 liters at rest to 20 liters at fast gallop. The respiratory quotient (RQ) (volume O_2 consumed/volume CO_2 expired) is used to determine the relative contribution of fat and carbohydrate to muscle metabolism, and is very useful in determining which type of metabolism is providing the major source of energy. It is regularly used in studies with human subjects, but is rarer in equine due to the problems associated with gas collection while the animal is in motion.

Influence of Exercising on Digestibility and Performance

Although the digestibility of a given diet is an important factor influencing requirements of the animal, few studies have been conducted on the effects of exercise level on digestibility. Scheider and Flatt (1975) reviewed several studies conducted in France and Germany during 1885 to 1891, and concluded that moderate work or exercise does not greatly influence digestibility. Hintz (1982) compared digestibilites of complete pelleted diets when horses were walked 30 min daily or ridden for 6 h daily at 12.8 km/h. He found that the digestibility of DM was slightly higher in the ridden horses (72.6%), compared with the walked horses (70.7%). Orton et al. (1985) found that exercising horses by trotting them at 12 km/h for 1 h/d increased feed intake and digestibilities of DM, energy and CP. These findings are significant

because there is usually a negative correlation between level of feed intake and DM digestibility at high feed intakes. This has been shown in horses by Haenlien et al. (1966), Hintz and Loy (1966) and Ott (1981). Blaxter (1962) found this to be more marked in sheep fed high-fiber diets, compared with low-fiber diets. It has been suggested that this depression of digestibility was due to a decreased mean retention time of digesta and therefore less time for microbial fermentation of structural carbohydrates (Orton et al., 1987).

Exercise has been shown to increase the fat-free weight gain in rats (Oscai et al., 1973). These authors postulated that the increase in DM intake when animals were exercised was the reason for the weight gain.

Exercise increases the energy requirement of animals (NRC, 1978) which offsets the effects of increased DM intake. Pindak and Pilat (1976) looked at the effect of exercise on the food intake, growth rate and feed to gain ratio in cattle. They found that exercise increased DM intake and rate of growth, but not feed to gain ratio.

Studies involving the effect of exercise in swine have shown mixed results. Andya et al. (1972) showed an increased liveweight gain in growing pigs, while Peo et al. (1970), Mandigo et al. (1971); Perrin and Bowland (1977), and Hale et al. (1984, 1986) showed little or no benefit in finishing or breeding performance. Peo et al. (1970) reported greater backfat thickness for exercised pigs and Mandigo et al. (1971) reported that percentage lean cuts was significantly increased by exercise. Fitts et al. (1976) reported that exercise in minature pigs did not significantly affect weight or carcass composition. Murray et al. (1974) found that exercise had no effect on carcass weight or the weight

of internal organs in young pigs and that there was no difference in either rate of gain or feed consumption. This finding is in agreement with those of Weiss et al. (1975) and Hale et al. (1984).

In a later study Hale et al. (1986) found that exercise had no effect on carcass length, backfat thickness, loin muscle area or lean cuts, nor on conception rates in gilts. However they did find that there were fewer limb soundness problems with the exercised pigs, which is in agreement with Perrin and Bowland (1977) who found that exercise reduced the incidence of leg abnormalities in boars.

Orton et al. (1985) investigated the effect of dietary protein and exercise on rate of growth in young horses. They found that exercise increased rate of growth and offset a higher feed to gain ratio in young horses fed a protein-deficient diet (50 % of NRC requirements). They also found that exercise increased digestibility of DM, energy and CP. In a further study Orton and coworkers (1985) investigated the effect of exercise on digestibility of various nutrients. Using fractional markers they measured the flow rates of the liquid and the solid portions of the digesta. They found that there was an increase in the mean retention time of the liquid portion of the diet and a decrease in the mean retention time of the solid portion when horses were exercised by trotting for at 12 km/h for 1 h/d.

Use of Markers to Determine Digestibility in Equines.

In 1966 Haelein et al. investigated the use of chromic oxide as a fecal marker for digestibility studies in horses. They compared the use

of a fecal marker with total collection and found that estimations of digestibility and fecal flow, using twice daily rectal grab sample for 4 d, compared with estimates using a 10-d total collection provided the recovery of chromic oxide was accounted for. Chromic oxide has frequently been used as a fecal marker for digestibility studies in horses since then. Orton et al. (1985) compared digestibilities in horses using chromic oxide as a marker with acid insoluble ash, and compared both of these with a particulate marker (ruthenium-phenanthroline) and total fecal collection. They found that estimates of apparent digestibility based on acid insoluble ash were similar to estimates based on total collection but that those based on chromic oxide were significantly lower.

Digestion and Utilization of Dietary Fat.

Effect of Dietary Fat. The beneficial effects of dietary fat has been shown in poultry (Potter 1967) and swine (Greeley et al., 1964; Leibrandt et al., 1975). Dunkley et al. (1977) reported an increase in milk fat content and yield when protected tallow was fed to cattle. Feeding of a suitable fat source to dairy cattle can restore milk fat levels in situations in which feeding a high level of grain depresses milk fat, and addition of fat to fat-deficient diets increases both milk and milk fat production (Palmquist 1978; Sharma et al., 1978).

In sheep it has been shown that the reduction in cellulose digestion which occurs when fat is added to the diet can be offset by the addition of Ca which helps in the emulsification of the fat (Brooks et al. 1954). However Bradley et al. (1966) found that the addition of ground limestone

to the diet had no beneficial effect on weight gains of steers. The depression of cellulose digestibility from the addition of fat was more evident when the proportion of roughage exceeded 50%. (Brooks et al., 1954; Bradley et al, 1966). Perry et al. (1976) found that the addition of 3% feed grade fat in a dry diet depressed crude fiber digestibility in lambs. However, Esplin et al. (1963), reported no effect on digestibility of nutrients in steers fed 4% fat and Johnson and McClure (1972) reported that addition of 6% fat to a high grain diet in lambs did not affect crude fiber digestibility. Generally in ruminants the effects of added fat in the diet are not consistent, and the beneficial effects only seem to appear in animals fed high-grain diets or when protected tallow is fed.

Fat Digestion in the Equine The primary site for fat absorption in most species is the small intestine (Hintz et al., 1980). It has been suggested that the equine would not be able to digest dietary fat due to the absence of a gall bladder. However it was shown by Swenson (1977) that horses continously secrete bile directly into the intestine and thus are fully capable of fat digestion.

Haenlein et al (1966) found that the digestion coefficients for crude fat of alfalfa pellets , wafers and loose hay, was -23,-14, and -26% respectively for horses, as compared with 30, 20, and 34% respectively for sheep fed the same forages. Cattle fed the alfalfa pellets and loose hay had respective crude fat digestibilities of 40 and 35%.

Vandernoot and Gilbreath (1970) found the crude fat digestibilities of orchardgrass, alfalfa, timothy, and bromegrass hays, were 47, 31, 49, and 40%, respectively, in horses, as compared with 53, 48, 61, and 57%,

respectively for steers fed the same forage. It has been suggested that one reason for the low apparent digestibility of crude fat or ether extract in horses is a high endogenous secretion of fat or ether soluble products in the feces, referred to as metabolic fecal fat (Armsby, 1922; Bryant, 1969). Bryant (1969) determined that the horse secreted approximately 83.3 g metabolic fecal fat per day, as compared with 1.5g in humans (Wolleager et al. 1953), 1.76 g in cats (Hill and Bloor, 1922), .51 g per day in dogs (Sperry and Bloor, 1924) and .11 g in rats (Carroll, 1958). Of course all the other species are considerably smaller in body weight than the horse, and have considerably lower total G.I. tract volume.

Rich et al. (1980) investigated the digestibility and palatability of vegetable, animal and blended fats in the equine. They found that the apparent digestibility of corn or peanut oil was higher than that of inedible tallow or blended fat. The true digestibility was not significantly different for the four kinds of fats. McCann et al. (1987) found that addition of 15 % corn oil to the diet of ponies increased energy balance approximately 20% over basal. Corn oil had a digestibility of over 90% when added as 10% of the diet in equine (Bowman, 1980). Kane and Baker (1977) fed diets containing 15 and 30 % added corn oil to ponies. They found that the addition of oil improved the digestibility of ether extract from 86.2 % (basal diet) to 91.5 (15 % added oil) and 93.5 % (30% added fat). They also reported that the digestible energy, metabolizable energy and net energy increased with the increasing fat content of the diet.

Utilization of Fat in the Equine Theoretically, feeding high levels of fat to equines during training could condition horses to use body fat more efficiently, therefore horses adapted to using fat would be able to use the fat to "spare" muscle glycogen and thereby delay the onset of exhaustion (Hintz et al., 1978; Hambleton et al., 1980). Slade (1975) fed a diet containing 12% fat (9% added corn oil) to horses that were being ridden for 67 km over mountainous terrain for 8 to 10 h. He found that the fat supplemented horses performed better and had higher blood glucose levels at the end of the ride than the horses fed the control diet. Maintenance of blood glucose levels during prolonged exercise is likely to be of benefit to endurance horses.

Pagan et al. (1987a, b) studied the effect of dietary energy source on blood metabolites. They found that horses fed high protein and carbohydrate diets relied more heavily on glucose metabolisim than horses on a high-fat diet. Furthermore, horses fed very high carbohydrate diets had higher muscle glycogen levels and higher blood lactates earlier in the exercise. One horse being trained on a high-carbohydrate diet 'tied up' badly after exercise. The same horse, when switched to a diet containing added fat, was able to complete the exercise test without a rise in serum creatine phosphokinase or any signs of tying up. All the horses relied more on fat metabolism towards the end of a long slow exercise period, but the horses fed added fat started to use fat sooner. During a short intense workout none of the horses used fat to any significant extent as measured by RQ. However as these horses were not conditioned to any extent the delay in mobilization and utilization may have been due to lack of fitness.

Webb et al. (1987) and Myers et al. (1987) investigated the effect of adding fat to the diet of race and cutting horses. They found that horses fed fat had a lower reliance on anaerobic metabolism (lower blood lactate and higher blood glucose) but they concluded that the horses fed carbohydrate may have been borderline glycogen deficient, because they could not maintain blood glucose homeostasis. However the cutting horses (who work anaerobically) had a slower recovery time and higher blood lactate when 10% fat was added to the diet. Presumably, the addition of fat did not help the horse during anaerobic work which is in aggrement with the findings of other workers.

There is usually a lag time between onset of exercise and mobilization of fat reserves and in very short duration exercise there simply is not enough time to mobilize, absorb and utilize FFA. The degree of fat utilization during a given exercise period will therefore depend not only on the duration and intensity of the exercise but also on the duration and efficiency of the pre- exercise warm up.

Chapter 2

JOURNAL PAPER 1

Effect of Exercise Training on Digestibility and Physiological Measurements in Equines

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ABSTRACT

The effect of exercise on apparent digestibility was studied by training a group of horses through a 60-d course of exercise training, then comparing their ability to digest various nutrients with a similar group of horses which were fed on the same diet but were not conditioned. The horses were exercised under saddle at walk, trot, canter and gallop. Apparent digestibility was determined by using chromic oxide as a marker. All horses were administered a standard exercise test on an equine treadmill. The trained horses had lower heart rate and blood lactate levels than the untrained controls for the same intensity and duration of work. Heart rate responses and blood lactate levels indicated that

exercised horses were fitter than unexercised controls. Conditioned horses had increased apparent digestibilities of DM ($P<.1$) (64.4 vs 59.7%), (ADF)($P<.1$) (36.1 vs 22.6%), and CP ($P>.05$) (68.7 vs 63.2). There were trends for increased digestibilities of neutral detergent fiber (NDF), cellulose and energy in the trained group.

Introduction

The rise in value and interest in the various equestrian sports and a burgeoning international industry in athletic events involving equine have resulted in increased interest in the equine as an athelete, which has emphasised a need for an increase in research into better methods of training and maintaining equines for athletic endeavors. While the benefits from this research have begun to affect the world of equestrian sports there are still large gaps in our understanding of the equine as an athelete, particularly in the region of nutrition. Part of the reason for this has been due to the fact that most research has been a spill over from the work in human athletics, which has produced a marked rise in standards of human sports. Horses may resemble humans in some areas of physiology but they are different in digestive physiology and nutritional requirements.

Although research in equine nutrition has intensified during recent years, little work has been done on the effect of strenuous exercise training on nutritional requirements, nor has the effect of fitness

training and exercise on efficiency of digestion been extensivly studied. Hintz (1982) compared digestibilities of complete pelleted diets when horses were walked 30 min/d or ridden for 6 h daily at 12.8 km/hr. He found that DM digestibility was slightly increased in ridden horses (72.6%), compared with the walked animals (70.7%). The present study was conducted to determine the effect of exercise training or conditioning on nutrient digestibility, and to determine the effect of a standard exercise test on certain physiological parameters indicative of fitness.

Experimental Procedure

Eight mature geldings of light horse breeding (Saddlehorse, Morgan, Quarter horse and Thoroughbred) with an average body weight of 478 kg and ranging in age from 3 to 10 yr were used in this experiment (appendix 1, table 1).

Diet The diet consisted of 47% ground orchardgrass hay, 36% ground corn grain, 15% sugarcane molasses, .5% trace mineralised salt and 1.5% limestone (table 1). The diet was calculated to provide 4.4 Mcal gross energy per kg and was fed to supply the NRC (1978) requirement for mature horses at light to medium work. The feed was given as a complete diet, and was mixed weekly in a vertical mixer. The orchardgrass hay was chopped in a high speed hammer mill through a 5.0 cm screen. The corn grain was ground through a 5.0 cm screen. The feed was stored in grain sacks stacked on a pallet and was weighed for each animal at time of feeding. Feed consumption, per animal per day, is given in appendix 1, table 2.

TABLE 1. Composition of Diet

Item	Value
Ingredient composition,% [a]	
Ground orchard grass hay	47.0
Ground corn grain	36.0
Sugarcane molasses	15.0
Limestone	1.5
Trace mineralized salt	.5
Chemical composition,%	
Dry matter	85.3
Crude protein[b]	10.5
Neutral detergent fiber[b]	50.4
Cell contents[b]	49.6
Acid detergent fiber[b]	23.9
Cellulose[b]	16.2
Gross energy, Mcal/kg[b]	4.4

[a] As fed basis
[b] Dry matter basis

The horses were fed at 2% of body weight to maintain body weight. They were weighed at 2 wk intervals (table 3 appendix 1) and the amount fed adjusted accordingly.

Water was availble from automatic waterers at all times, both in the stalls and dry lots.

Training. The horses were paired according to weight and breeding, one of each pair being alloted at random to the exercised group the other served as a control. The exercised horses were ridden 5 d/wk for lengthening periods of time and at increasing speeds, (table 4, appendix 1) beginng with .5 h walk which increased up to 2-h walk, trot, canter and gallop. The horses were ridden around the fields of the experimental farm and were cantered or galloped in an enclosed area using a stopwatch to time the length of the workouts. Heart rates were measured using a heart rate monitor[1] with surface adhering electrodes. All horses had a rectangle of hair clipped out under the saddle and over the sternum (under the girth) for placement of the electrodes, to ensure proper contact with skin.

Initially, the plan was to use a heart rate monitor to measure daily heart rate response to the work for each animal. Since there were only three monitors, it was necessary to rotate them around the horses in order to measure all animals, thus,it was not possible to measure each horse each day. Furthermore, not all the riders were able to handle both the horse and the monitor, particluarly at the higher speeds, so those riders who could, had to rotate around the horses during the gallops. Mean

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[1] Equine heart rate computer, EQB,Doe Run Rd, Unionville, Pa.

heart rates for the horses at various paces are given in table 2. The heart rates at the various paces were compared to rates given in the literature in order to ensure that each animal had an adequate and comparable work load. Resting (in stall) heart rates were taken for all horses at weekly intervals (table 5, appendix 1). During the time that the exercise group were being ridden, the control horses remained in the stalls.

Management. The horses were maintained in 3.2 X 2 m stalls during the day and were turned out into dry lots at night. The horses were fed three times per day, (0700, 1200 and 1700 h). All horses were wormed with ivermectin[2] and were weighed prior to the start of the experiment. All the animals were accustomed to working under saddle. Two weeks before the experiment began, the horses were turned out together in a dry lot at night for adaptation to other horses and to the pen. During this period they were fed in their stalls three times per day. No feed was given when the animals were in the dry lot. Trace mineral salt and water were availible ad libitum while in the dry lot. Whether ridden or not, all horses were groomed daily. Any minor wounds or ailments were treated. Fortunately, there were no major health problems with any of the animals. The four exercised horses were shod in front only and the shoes were replaced and the feet trimmed at 6-wk intervals (at the start and once during the trial).

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[2] Zimerectin, Farnham group of Co, Omaha, Nebraska.

TABLE 2 Mean Heart Rates for the Various Paces.

Horse	Paces				
	Rest[a]	Walk[b]	Trot[b,c]	Canter[b,d]	Gallop[b,d]
	beats/min				
2	34	85	105	180	200
3	34	75	117	145	200
5	36	85	130	180	195
8	38	85	125	165	210

[a] Taken in stall no rider or tack
[b] Taken with rider in saddle
[c] Mean rate for end of trot session
[d] Mean for end of canter or gallop last four sessions

Digestion Trial. A digestion trial, using chromic oxide (Cr_2O_3)at .5% of the diet as the marker was conducted before and after the training period. The horses were fed the Cr_2O_3 in the diet for a 7- d preliminary period, after which rectal grab samples of feces were collected at 0600 and 1800 h for 5 d. Feed samples were taken at each feeding during the collection period and frozen within 30 min. During the preliminary period the maximum intake for each animal was determined and during the collection period each animal was fed only what it would clean up, so there were no refusals.

Each collection sample of feces was frozen within 30 min. Later the samples were thawed and a 50 g portion taken from each sample. These were pooled by animal, and subsampled. One portion was frozen for N determination and two samples were dried at 100 C, ground in a hammer mill (1mm screen) and stored for analysis of cell wall components and energy.

Standard Exercise Test. After the training period the horses underwent a Standard Exercise Test (SET) using a treadmill.[3] The treadmill was on a 10% incline and could be run at speeds up to 216 m/min. Each horse was introduced to the treadmill over a 2-wk period. Only six of the eight horses would work on the treadmill, so there are only six observations for the SET. Of the six horses, three had not been exercised and there was concern that if a standard SET (Persson, 1983) was used these animals could be injured, therefore a modified SET was used (table 6, appendix

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[3] Equine treadmill, Jetline Treadmills, Sand Lake, MI.

1). The purpose of the SET was to establish differences in heartrate and blood lactate responses to exertion in trained and untrained horses to examine if there was a measurable difference associated with the degree of physical conditioning. Another purpose was to become accustomed to the use of a SET as a measurment of physiological response to exercise stress. A heartrate monitor was used during the SET to measure heart rate (table 8, appendix 1).

The animals were taken out individually from their stalls and brought to the testing area there the heart rate monitor was attached and the pre-exercise rate was measured, a blood sample was taken by venupuncture, one into a heparinized tube for plazma and one into a plain tube for serum. The blood samples were stored on ice.

The horses were put on the treadmill, the speed was set for a slow walk (80 m/min) and this speed was maintained for 3 min. There was a time lapse between measuring the heart rate and increasing the speed. Measurement of time at a given speed was started when that speed was achieved. The test used was a step test which meant that the speed was increased in a step-wise manner from the start to the finish of the exercise. After 3 min the speed was turned up to 162 m/min which represented a slow trot. After 10 min the speed was increased to a faster trot (186 m/min). Heart rate measurements and blood samples were taken as shown in table 6, appendix 1. After 17 min the treadmill was stopped, the animal was removed, the heart rate was measured and another blood sample taken by venupuncture. The heart rate was measured and another blood sample taken at 5 min after the end (22 min) and again at 10 min (27 min).

After the end of the SET the horses were returned to their stalls and were fed. All the animals were fasted from the evening before until their SET to prevent interference from absorbed carbohydrates affecting glucose or lactate levels.

Analysis of Blood Samples. The blood samples were stored on ice until they were centrifuged and the plazma separated and frozen, or were allowed to clot, then centrifuged and the serum frozen. Heamatocrits were measured in duplicate on each whole blood sample[4] prior to centrifugation (table 9, appendix 1). A 1 ml portion of whole blood was deproteinised with 9 ml of tungstic acid, filtered and the filtrate was assayed for lactic acid.[5]

Analysis of Feed and Fecal Samples. Frozen feed and fecal samples were pooled by animal, a subsample was removed and frozen, and the remainder was dried in a 100 C forced air oven, allowed to equilibrate to atmospheric moisture and ground through a Wiley mill using a 1 mm screen.

Feed and feces were analyzed for DM, N, ether extract (A.O.A.C., 1980), NDF, cell solubles and ADF (Goering and Van Soest, 1970). Nitrogen was determined on the wet feces. Energy determinations were by bomb calorimetry. Chromic oxide levels were determined spectrophotometrically after acid digestion, using the method of Hill and Anderson (1958).

Statistical Analysis The design was a randomized block design with treatments allotted at random within blocks. The data were tested by

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[4] Microhaematocrit II, Adams, Fisher Scientific , Raleigh, NC.

[5] Sigma kit No 826-uv, Sigma Chemical Co, St Louis, Mo.

analysis of variance using the general linear model (GLM) procedure of SAS (SAS,1982). All means reported were least squares means.

Treatment (conditioning) was the only effect in the model for the digestion trial. The data for the exercise trial were analyzed with treatment and time in the model. Correlation coeffecients between time, heart rate and lactate were computed using the procedure of SAS.

Results and Discussion

Apparent Digestibility. Conditioned horses showed higher apparent digestibility for crude protein (68.7 vs 63.2%) ($P<.05$), dry matter (64.4 vs 59.7%) ($P<.1$) and ADF (36.1 vs 22.6%) ($P<.1$) (table 3). They also tended to have higher digestibility for cell contents (78.69 vs 76.36 %), NDF (61.2 vs 58.0%), cellulose (38.4 vs 28.1%) and energy (64.2 vs 59.0%). Individual values are given in table 9 appendix 1. The increase in apparent digestibility with exercise training is in agreement with the results of Hintz et al. (1982) who found increases in digestibility of the fiber, dry matter, and crude protein fractions of a complete pelleted diet, when horses were ridden for 6 h daily at 12.8 km/h (213 m/min or steady trot), 5 d/wk as compared with horses that were walked for 30 min daily.

Orton et al. (1985), in a study on the effects of exercise on digestibility of various nutrients, used several markers, some of which were carried in the fluid portion of the diet and others in the solid portion. They reported an increased mean retention time for the fluid

portion of the diet. This portion contains the potentially more digestible fractions, hence increased retention time would would increase exposure to digestive enzymes and more time for absorption in the small intestine.

In man, cycling exercise appears to have little effect on the rate of fluid movement out of the G.I.tract (Costill et al. 1974). However, Neufer et al. (1986) looked at the effect of running exercise on the rate of gastric emptying in man, using various solutions of sugars and plain water. They found that exercise had the effect of increasing rate of gastric emptying, possibly due to increased mechanical movement of fluid within the stomach. The sugar solutions were delivered into the duodenum faster following 15 min running exercise, compared with 15 min seated rest. Thus, the fluid was made accessible to the intestinal mucosa at a faster rate, which might give some help to the digestion and absorption of the carbohydrate portions of the meal, but would not help explain the increased protein digestibility.

If the solid portion were also retained longer this would allow increased time for the activity of hind gut microbes to breakdown the fibrous portions. However, Orton and coworkers (1987) reported a decreased retention time for the solid portion of the diet with exercise, so there must be some other reason for the increase in fiber digestion in the present study. In this experiment the marker (Cr_2O_3) used marks both the particulate and fluid portions of the digesta. Thus, it was not possible to differentiate between fluid and solid portions.

TABLE 3 Effect of Exercise on Apparent Digestibility.

Component	Treatment	
	Control	Exercise
	-------%-------	
Dry matter[a]	59.7	64.4
Crude protein[b]	63.2	68.7
Neutral detergent fiber	58.0	61.2
Cell contents	76.4	78.7
Acid detergent fiber[a]	22.6	36.1
Cellulose	28.1	38.4
Energy	59.0	64.2

[a] Means differ (P<.1)
[b] Means differ (P<.05)

In equine most of the separation of between the two digesta phases occurs in the hind gut due to selective retention of larger particles (Argenzio et al,. 1974). Rates of passage of both digesta phases in horses are generally faster than in ruminants, and as a consequence apparent digestibilities, particularly of fiber, are usually lower in equine (Janis, 1976).

It has been shown in man that exercise increases the body core temperature and general body metabolisim (Bielinski et al., 1985), and that submaximal exercise increases rate of blood flow through the splancnic region. This increase in body temperature and metabolic rate lasted for up to 4-h post exercise. A rise in body temperature of 1 to 2 C may enhance rate of microbial activity (Clarke and Bauchop, 1977) and thus could increase fiber digestibility. Also, increased rate of blood flow though the splancnic region, particularly the liver, could result in an increased uptake of VFA.

It has been shown in ponies (Hintz, 1983) that the liver can use one of the VFA (propionate) as a gluconeogenic precursor. If the rate of exercise is submaximal there is no great shunting of blood out of the splancnic regions, to the muscles or to the subcutaneous region for temperature regulation (Rose, 1982) Furthermore, at submaximal levels of exercise there is increased utilization of free fatty acid as fuel for liver and muscle activity (Snow, 1983). Thus, it is possible that the increased rate of blood flow through the splancnic region will increase the rate of absorption and subsequent utilization of the by-products of microbial fermentation.

Standard Exercise Test. Heart rate was lower in the conditioned horses at rest (38 vs 40 beats/min $P<.05$), and tended to be lower at walk (96 vs 104 beats/min), 5 min (trot)(178 vs 185 beats/min), 10 min (180 vs 184 beats/min) and at 15 min (169 vs 190 beats/min) (figure 1). The conditioned horses had a lower heart rate at 18 min (128 vs 135 beats/min) ($P<.05$) during the recovery period, 22 min (5 min recovery) (75 vs 80 beats/min) and 27 min (10 min recovery) (60 vs 62 beats/min). Heart rate is linear to work load on a treadmill expressed as speed up to a heart rate of 210 beats/min (Persson 1983). Heart rates above 250 are considered indicative of extreme stress in the horse (Persson, 1983). None of the horses, whether conditioned or not, showed a heart rate above 210 beats/min during the test, which by the criterion of Persson (1983) would indicate that the exercise test was not sufficiently strenuous and did not fully exert the horses, particularly the conditioned horses. Future exercise tests would need to be either at faster speeds and/or for for longer time periods. Individual results for each horse are shown in table 7, appendix 1.

Blood Lactate There was no difference in blood lactate between the two groups before the test (1.22 vs 1.23 mmol/liter) (figure 2). The conditioned horses had lower blood lactate levels immediately following the exercise test (1.73 vs 4.65 mmol/liter, $P<.05$), at 5 min post exercise (1.59 vs 3.65 mmol/liter, $P<.05$) and 10 min post exercise (1.58 vs 3.27 mmol/liter, $P<.05$). None of the conditioned horses showed blood lactate levels in excess of 4.0 mmol/liter or onset of blood lactate accumulation (Persson, 1983), which indicated that they had sufficient cardiovascular fitness for the work load. Two of the unconditioned horses had blood

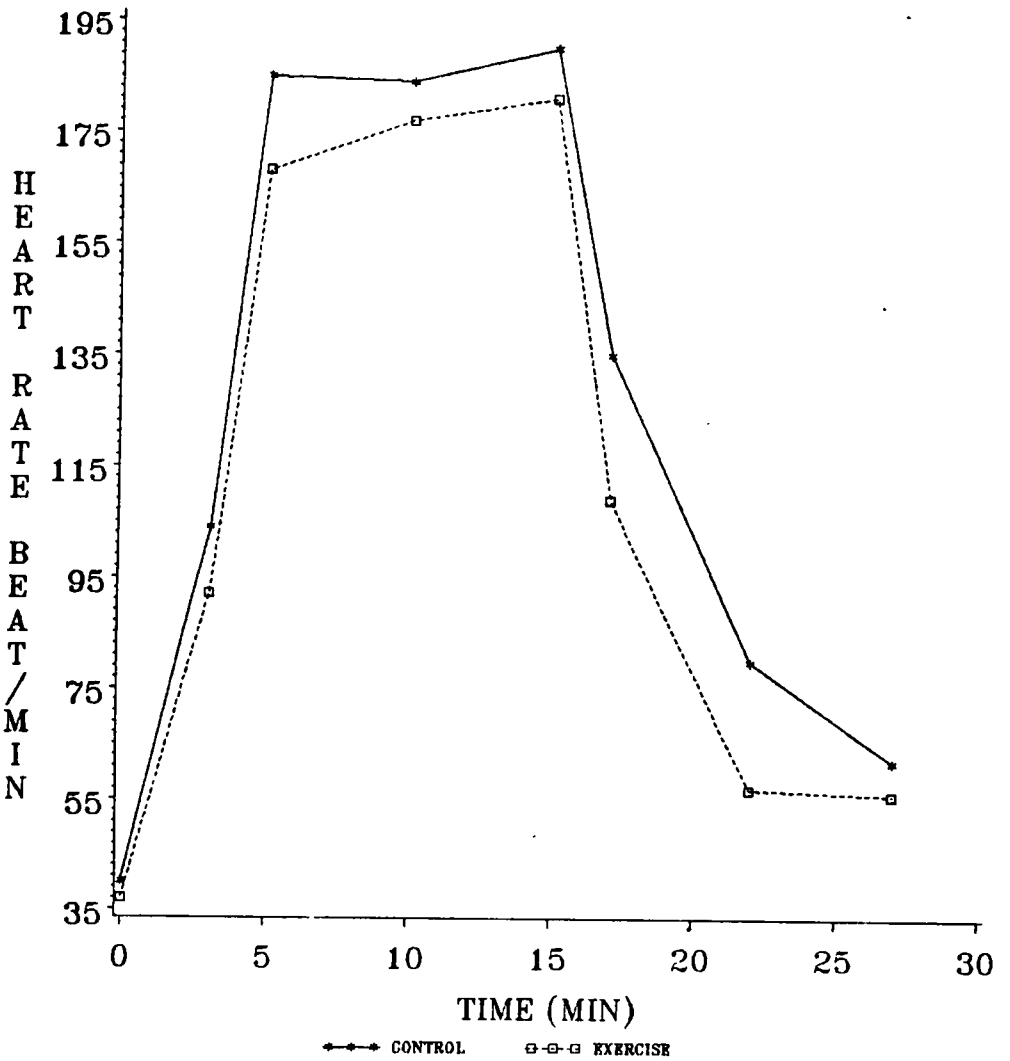

Figure 1. Heart Rate for Control and Exercised Horse Experiment 1.

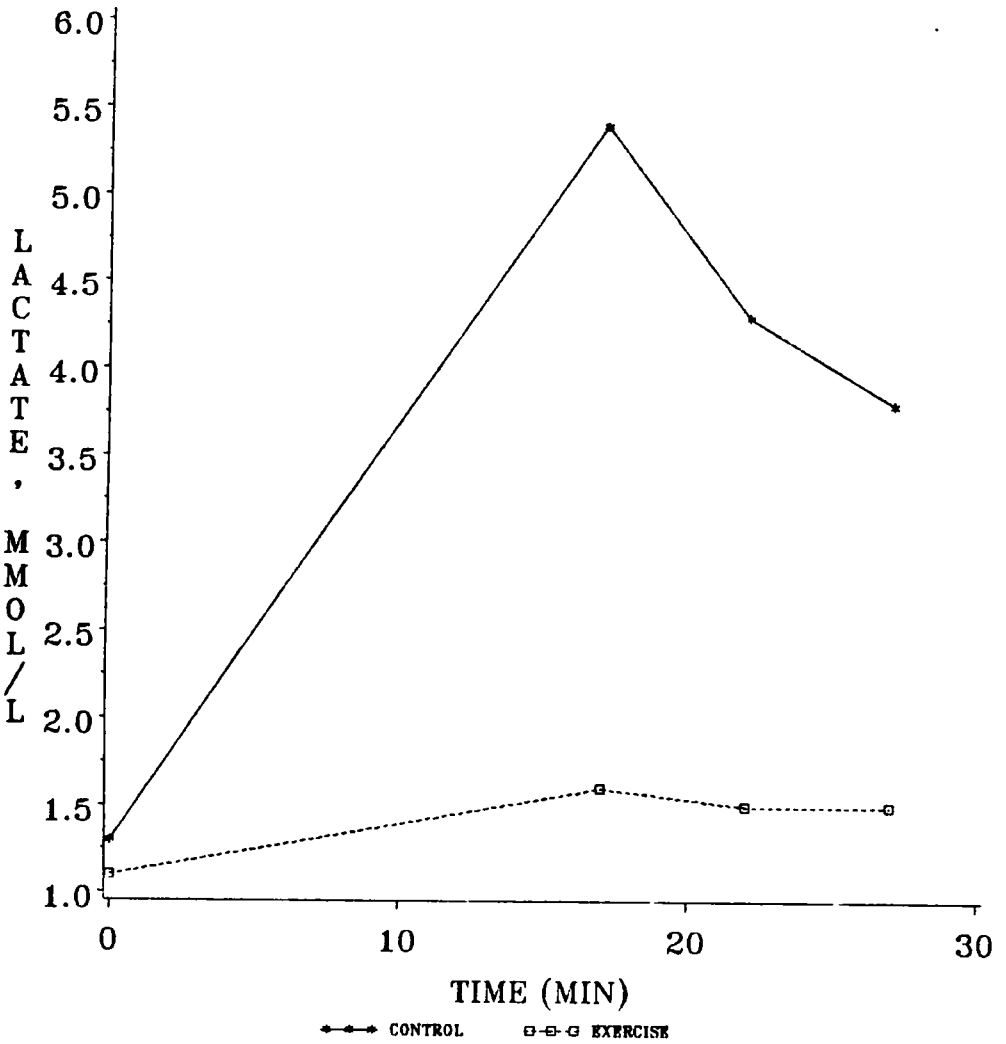

Figure 2. Blood Lactate Concentrations for Contr ol and Exercised Horses. Experiment 1.

lactate levels in excess of 4 mmol/liter, both immediately after exercise and 5 min after cessation of exercise. Therefore, the unconditioned horses were much less fit than the exercised horses. Individual results for each horse are shown in table 8, appendix 1.

Packed Cell Volume. All horses showed an increase in packed cell volume (PCV) with exercise, which is normal in the horse (Blackmore and Brobst, 1975) There was no difference between the conditioned horses and the control group (table 4). Packed cell volume values for all horses were within the normal range for equine (Blackmore and Brobst, 1975). Individual results for each horse are shown in table 8, appendix 1.

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Table 4. Packed Cell Volumes.

Treatment	Time, min			
	0	17	22	27
	----------%			
Control	36	44	41	38
Exercise	32	46	40	40

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CHAPTER 3

JOURNAL PAPER 2

PHYSIOLOGICAL EFFECTS OF EXERCISE AND DIET ON METABOLISM IN THE EQUINE

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ABSTRACT

An experiment was conducted to evaluate the effects of exercise on nutrient utilizaton and to determine the efficacy of dietary fat as an energy source for the exercising horse. Supplying calories in the form of additional fat, depressed ($P<.05$) apparent digestibilities of dry matter (DM), cell contents, energy, and neutral detergent fiber (NDF) in unconditioned horses. Addition of fat increased apparent digestibility of ether extract (89.2 vs 65.6 %, $P<.05$). In conditioned horses the addition of fat lowered ($P<.05$) the apparent digestibility of DM and NDF. The addition of fat increased ($P<.05$) the digestibility of ether extract in conditioned horses. Conditioning increased ($P<.05$) apparent digestibility of crude protein and energy. There were no differences in physiological parameters used for assessing fitness (heart rate, blood lactate, respiration rate and body temperature), between horses fed a diet containing 14% added fat and no added fat. There was no difference in blood glucose levels,

blood urea N (BUN) or creatin phosphokinase (CPK) between horses fed the two diets.

Introduction

It has been estimated that there are 5 million horses in the U.S. (A.H.C.,1987). Many of these are used in strenous sports, such as racing, horse trials, show jumping, endurance riding and driving. Improved performance has been achieved in human athletes during the last 40 yr, but despite active breeding programs, better training and improvements in veterinary medicine, comparatively few advances have been made in equine performance. High levels of athletic performance require an increased input of dietary energy.

The traditional method of increasing the availible energy content of equine diets is to increase the amount of starch in the diet, usually by increasing the amount of grain. Diets containing over 50% concentrate can lead to a variety of problems including digestive problems, such as colic; and circulatory conditions, such as laminitis; which can lead to founder (Meacham, 1987). A high level of dietary carbohydrate has also been cited as a factor in conditions such as rhadomyolysis (Hintz 1982), and yet the energy required for strenous activities must be supplied. Recent work has shown the horses can digest and utilize dietary fat (Hambleton et al., 1980; Bowman, 1983; Hintz, 1983; McCann et al., 1987), but there is little information on whether supplying some of the energy needs of working

horses as fat has a beneficial or deleterious effect on fitness and exercise conditioning parameters.

The present study was conducted to further investigate the effect of exercise on nutrient digestibility and evaluate the efficacy of fat as an energy source for the exercising horse using a standard exercise test on a treadmill.

Experimental Procedure

Eight mature geldings of light horse breeding (Saddlehorse, Morgan, Quarter horse and Thoroughbred) with an average body weight of 478 kg and varying in age from 3 to 10 yr were used. The group of eight geldings were paired according to weight and breeding (table 10 appendix 2). One member of each pair was randomly assigned to the high-fat diet group and one to the control group.

Diets The control group were fed a diet of ground orchardgrass hay, ground corn grain and sugar-cane molasses (table 5), and the experimental group were fed a diet calculated to be isocaloric with respect to gross energy, containing ground orchard-grass hay, oathulls, molasses and 14% added fat.[6] The fat used was based on choice white grease in a dextrose-casein base which supplied 80 % fat.

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[6] Merricks dry fat 48-0, Merricks, WI.

TABLE 5. Composition of Diets. Experiment 2.

Item	Diet	
	Control	Fat
Ingredient composition, %[a]		
Ground orchardgrass hay	38.0	55.0
Ground corn grain	44.0	0
Oathulls	0	13.0
Sugarcane molasses	15.0	15.0
Fat[b]	0	14.0
Trace mineralized salt	.5	.5
Limestone	2.5	2.5
Chemical composition,[c]		
Dry matter, %	85.0	70.1
Crude protein %[c]	10.2	10.4
Neutral detergent fiber %[c]	47.8	41.0
Cell contents %[c]	52.2	59.2
Acid detergent fiber %[c]	21.3	25.8
Ether extract %[c]	2.1	14.1
Gross energy, Mcal/kg[c]	4.25	4.6

a As fed basis
b Choice white grease in a dextrose/casein base
c Dry matter basis

The feed was stored in grain sacks stacked on a pallet and was weighed out for each animal at time of feeding.

Training. The horses were ridden 5 d/wk for lengthening periods of time and at increasing speeds, as in experiment 1, (table 3, appendix 1) begining with .5 h walk which increased up to 2 h walk, trot canter and gallop. The horses were ridden around the farm fields during exercising and were cantered or galloped in an enclosed area using a stopwatch to time the length of the workouts.

Heart rates were measured using a heart rate monitor[7] which had adhesive electrodes. A rectangle of hair was clipped under the saddle and over the sternum (under the girth) from each horse, for placement of the electrodes. Mean heart rates for horses were similar to those found in experiment 1 (table 2).

Management. The horses were maintained in 3.8 m X 2 m stalls during the day and were turned out into drylots at night. The horses were fed three times per day (0700, 1200 and 1700 h).

All horses were wormed with ivermectin[8] and were weighed prior to the start of the experiment. All the animals were accustomed to working under saddle. Two weeks before the experiment began, the horses were turned out together in a drylot at night to aquaint them to each other and to the pen. During this period they were fed in stalls, three times per day. No feed was given when the animals were

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[7] Equine Heart rate computer; EQB, Doe Run Rd, Unionville, Pa.

[8] Zimerectin; Farnham group of Co, Omaha, NA.

in the dry lot, to reduce fighting and because individual intakes could not be controlled. Trace mineralised salt was available while in the drylot.

The horses were initially fed at 2% of body weight. They were weighed at 2 wk intervals and the amount fed was adjusted to maintain bodyweight. The dry matter intake by day is given in table 11, appendix 2. Weights for individual horses are given in table 12, appendix 2. The diet was weighed into individual buckets at each meal and then placed in a round corner feeder, fitted with an anti-spill ring, attached to the wall. Water was availible from automatic waterers at all times, both in the stalls and drylots.

All animals were turned out together at night in the same pen. The horses were groomed daily and any minor wounds or ailments were treated. There were no major health problems with any of the animals. The horses were shod in front only and the shoes were replaced and the feet trimmed at 6 wk intervals, (at the start and once during the trial).

Digestion Trial. A digestion trial, using chromic oxide (Cr_2O_3) (.5% of diet) as the marker, was conducted before and after the training period. The horses were fed the diet with Cr_2O_3, for a 7-d preliminary period, after which rectal grab samples of feces were collected at 0600 and 1800 h for 5 d. Feed samples were collected at each feeding, and subsequently pooled and subsampled. During the preliminary period the maximum intake for each animal was determined

and during the collection period each animal was fed only what it would consume, hence, there were no refusals.

Samples of feces were frozen within 30 min. Later, the samples were thawed, 50 g from each collection were pooled by animal,and subsampled.

Analysis of Feed and Feces. Frozen feed and fecal samples were pooled by animal, and a subsample was removed and frozen while the remainder was dried in a 100 C forced air oven, allowed to equilibrate to atmospheric miosture and ground through a Wiley mill using a 1 mm screen.

Feeds and feces were analyzed for dry matter, N, ether extract (A.O.A.C.,1975), NDF, cell contents and ADF, (Goering and Van Soest, 1970). Nitrogen was determined on the wet feces and energy determinations were made on dry feed and faeces by bomb calorimetry. Chromic oxide was determined spectrophotometrically using the method of Hill and Anderson (1958) after digestion with nitric and perchloric acids.

Standard Exercise Tests. Prior to the start of conditioning the horses were subjected to a standard exercise test on the treadmill for 25 min (SET 1). Horses which had not been previously exposed to the treadmill were given enough training to accustom them to trotting on it, each animal was trotted on the treadmill at least twice. The protocol for the SET is given in table 13, appendix 2.

It was considered desirable to be able to obtain blood samples during the actual period of exercise, so each horse was fitted with

an indwelling jugular cannula, fitted with a stopper and taped to the neck. Catheterisation was done at least 2 h prior to the SET.

The heart rate, temperature and respiration rate were measured in the stalls prior to bringing the animals to the test area. The pre-exercise blood samples were taken at the test area in sight of the treadmill. The animals were put on the treadmill, and the speed turned up to a medium walk, (81 m/min for 5 min), the speed was increased to 162 m/min for 10 min, then the speed was increased to 174 m/min for 5 min, then up to 187 m/min for the last 5 min. Blood samples and heart rates were taken prior to the test, and after 5,8,13,15,18 and 22 min of the test. At 25 min the animals were removed from the treadmill and blood samples and heart rates were taken immediately on removal, after 5 min and 10 min after removal.

Respiration rates were counted using a stopwatch as soon as possible after removal from the treadmill and after 5 min of recovery. Temperatures were taken before the exercise test and again at the end of exercise using a digital thermometer. Average ambient temperature for the 2 d was 14 C and relative humidity was 20%.

After the conditioning period, a second SET (SET 2) was run following the same procedure as SET 1, with the exception that the speeds were increased to allow for the greater fitness of the horses, no blood sample was drawn at 5 mins (walk) and only heart rate was measured. At the end of the exercise, two heart rates were measured, once just before the treadmill was shut off and again as soon as the animals had decended from the treadmill (approximately 1 to 2 min).

Table 13 appendix 2 shows a protocol for SET 2. Mean ambient temperature for the 2 d was 29 C, and relative humidity was 80%.

Analysis of Blood Samples Blood samples were divided into two heparinized tubes and one non-heparinized tube, which were placed on ice. One heparinised tube containing whole blood was used for lactate analysis,[9] lactates were read within 40 mins after the blood sample was taken. The other tube of whole blood was centrifuged within 30 min and the plazma was frozen for glucose analysis. Packed cell volumes were measured[10] on whole blood prior to centrifugation. The remaining tube was allowed to clot, centrifuged and the serum frozen for later analysis. Creatine phosphokinase levels were measured[11] on frozen serum. Blood urea N was measured on samples from SET 2 by the method of Coulombe and Favreau (1963).

Statistical Analysis. Data for the digestion trials in were tested by analysis of variance using the general linear model (GLM) procedure of SAS (SAS, 1982). All means reported were least squares means.

The data were analyzed as a 2 x 2 factorial. The main effects were SET and diet. The SET x diet interaction was tested. The data

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9 YSI Model 23L Lactate analyser, ELO Springs Instruments, American Scientific Product Distributors, Il.

10 MicrohematocritII,Adams,Fisher Scientific, Raleigh, NC.

11 Sigma kit No520, Sigma Chemical Co, St Louis, Mo.

were also analyzed separately for SET within treatment and for treatment within SET in the model.

Data for the exercise trial in experiment 2 were analyzed with diet, horse within diet, time and interaction between diet and time in model. This model was run separately for each SET.

Results and Discussion

Pre-Conditioning Digestion Trial. Prior to conditioning the horses fed a fat-supplemented diet had a lower apparent digestibility of dry matter (56.7 vs 67.3 % $P<.05$) (table 6). This is in contrast to the data reported by Rich et al. (1982), who found that addition of fats to the diet had no effect on dry matter digestibility in ponies. However in their studies fat was added to the diet, whereas, in the present study fat was substituted, with both the diets being isocaloric.

There was no significant difference in apparent digestibility of crude protein, but there was a trend towards lower digestibility for the high-fat diet (64.7 vs 66.6%). This in contrast to the data reported by Bowman et al. (1977), who found an increase in apparent crude protein digestibility in ponies fed a diet supplemented with corn oil, and that reported by Rich et al. (1982), who found a slight increase in apparent digestibility of crude protein in ponies supplemented with corn oil,

TABLE 6. Effect of Diet and Conditioning on Apparent Digestibility by Horses.

Component	Unconditioned		Conditioned	
	Control diet	High fat diet	Control diet	High fat diet
	----------%			
Dry matter[a]	67.3	56.7	70.4	60.7
Crude protein[b]	66.6	64.7	70.1	73.7
Acid detergent fiber	24.6	17.8	23.4	26.8
Neutral detergent fiber[a]	51.3	29.2	51.7	30.4
Cell contents[a]	82.0	75.6	80.6	76.7
Ether extract[a]	65.6	89.2	54.4	88.7
Energy[a, b, c]	71.8	61.2	70.4	65.6

a Diet effect ($P<.05$)
b Conditioning effect ($P<.05$)
c Conditioning x diet interaction ($P<.05$)

and a trend towards lower digestibility in diets supplemented with blended fat or tallow. Swift et al. (1948) and Summers et al. (1957) reported reduced crude protein digestibility in ruminants fed 2 or 3% added fat.

Digestibility of cell contents was less for horses fed the diet containing added fat (75.5 vs 82.0 % $P<.05$). Neutral detergent fiber digestibility was also lower for the diet containing fat (29.2 vs 51.3 % $P<.05$). Although there was no significant difference in digestibility of ADF, there was a trend for a lower apparent digestibility for the diet containing fat (17.8 vs 24.6 %). Rich et al. (1982), found that the digestibility of NDF was higher for diets containing no fat and corn oil, compared to diets containing blended fat and tallow. These workers also found a trend for lower digestibility of ADF in diets supplemented with blended fat and tallow. Brooks et al. (1954), Henderson (1973) and Kowalczyk et al. (1977), reported that addition of fat to ruminant diets deppressed cellulose digestibility. Perry et al. (1976), reported a reduced crude fiber digestibility in sheep fed a diet containing 3% feed grade fat. Rich et al. (1982), in a second trial, found that ADF digestibility increased with level of fat added to the diet, but suggested that the use of alfalfa hay as a forage source in the diet prevented the depression in digestibility due to the added fat. Brooks et al. (1954) found that the depression of fiber digestibility in sheep was prevented by addition of alfalfa ash. White et al. (1958) showed that the effect of alfalfa ash was probably due to its high

Ca content. In the present study grass hay was used, which is much lower in Ca than alfalfa.

The low digestibility of ADF found in this study for the high-fat diet, may also be partly due to the large content of oat hulls in the diet which was used to make the diets isocaloric. This, in combination with the poor quality grass hay, resulted in a slightly higher lignin content than the control diet (5 vs 4%).

Horses fed the diet containing added fat showed a higher apparent digestibility for ether extract than controls (89.2 vs 65.6 %, $P<.05$). These results are in agreement with those reported by Rich et al. (1982) and Kane and Baker (1977) who found that ether extract digestibility was higher in diets supplemented with corn oil, peanut oil, blended fat or tallow.

Energy digestibility was lower in the diet containing added fat (61.16 vs 71.77 % $P<.05$), which may have been a reflection of the high fiber content or of the lower digestible energy content. In most previous studies the addition of fat to the diet increased the apparent energy digestibility (Rich et al., 1982; Kane and Baker 1977; McCann 1987), but these diets were not isocaloric and the higher energy digestibilities may have been a reflection of the higher energy content of the fat- supplemented diets. Individual results are shown in table 14, appendix 2.

Post-Conditioning Digestion Trial. The conditioned horses showed a higher apparent digestibility for crude protein for the fat-supplemented diet, (table 6) (73.7 vs 64.7 % $P<.05$), compared with

unconditioned horses. Although the fat supplemented horses tended to have a higher apparent digestibility than the control animals (73.7 vs 70.1 %), after conditioning, the difference betweeen the two diets was not significant.

Conditioned horses tended to show a higher apparent digestibility of dry matter for both diets, compared with pre- conditioning values. The fat-supplemented group showed a lower apparent digestibility for dry matter (60.7 vs 70.4 %, $P<.1$) compared to controls.

Apparent digestibility of ADF tended to be higher after conditioning. The conditioned horses fed the high-fat diet tended to have a higher digestibility for ADF, compared with conditioned animals fed the control diet (26.8 vs 23.4 %). Apparent NDF digestibility of both diets did not change with conditioning, nor did conditioning affect the apparent digestibility of cell contents and ether extract for horses fed either fat supplemented or control diet. Horses fed the diet containing fat had higher apparent digestibility for ether extract (88.7 vs 54.4 % $P<.05$). Conditioning increased apparent digestibility of energy only for the high-fat diet (65.57 vs 61.60 %, $P<.05$). There was a conditioning x diet interaction ($P<.1$).

In this experiment the conditioning effect on apparent digestibility was less than in the previous experiment (Chapter 3). This may have been due to slightly less efficient training. Individual results are given in table 15, appendix 2.

Standard Exercise Test, Pre-conditioning. Initially, the fat supplemented horses showed a slightly but not significantly higher body temperature (37.6 C) than the controls (37.1 C) and there was no trend after exercise (39 vs 38.9 C) (table 7). 17 C and relative humidity was 20%. Individual results by horse are shown in table 16, appendix 2.

The fat-supplemented horses showed a trend towards higher respiration rates prior to the exercise test (27 vs 22 breaths/min), immediately after the exercise test (119 vs 105 breaths/min), and 5 min post exercise (61 vs 51 breaths/min) (table 7). They also showed a trend towards a faster recovery at 5 min post exercise (79% vs 72 % of resting rate). Respiration rates were all within the normal range for equine and no horse showed an inverted heart/respiration rate ratio immediately following the SET nor at 5 min post exercise. Inverted heart/respiration rates are usually regarded as an indicator of respiratory distress and insufficient conditioning for the work done. Individual results by horse are shown in table 16, appendix 2.

Heart Rate. Horses fed the diet containing added fat tended to have a higher mean heart rate at 13,15,18 and 25 min and lower heart rate at rest, 5 min, 8 min, 30 min (5 min recovery) and 35 min (10 min recovery),(figure 3) but there was no significant difference between them (table 8). The mean resting heart rates were 47 beats/min for control and 49 beats/min for those fed added fat. Rates were well within the normal resting range for unconditioned horses (Snow, 1983).

TABLE 7. Pre-Conditioning Temperatures and Respiration Rates of Horses, by Diet. Experiment 2, SET 1

Item	Diet			
	Control	Added fat	Control	Added fat
	---temp, C---		breaths/min	
Pre-exercise	37.0	37.5	22	27
Post-exercise	39.0	39.0	105	119
5 min post-exercise			51	61

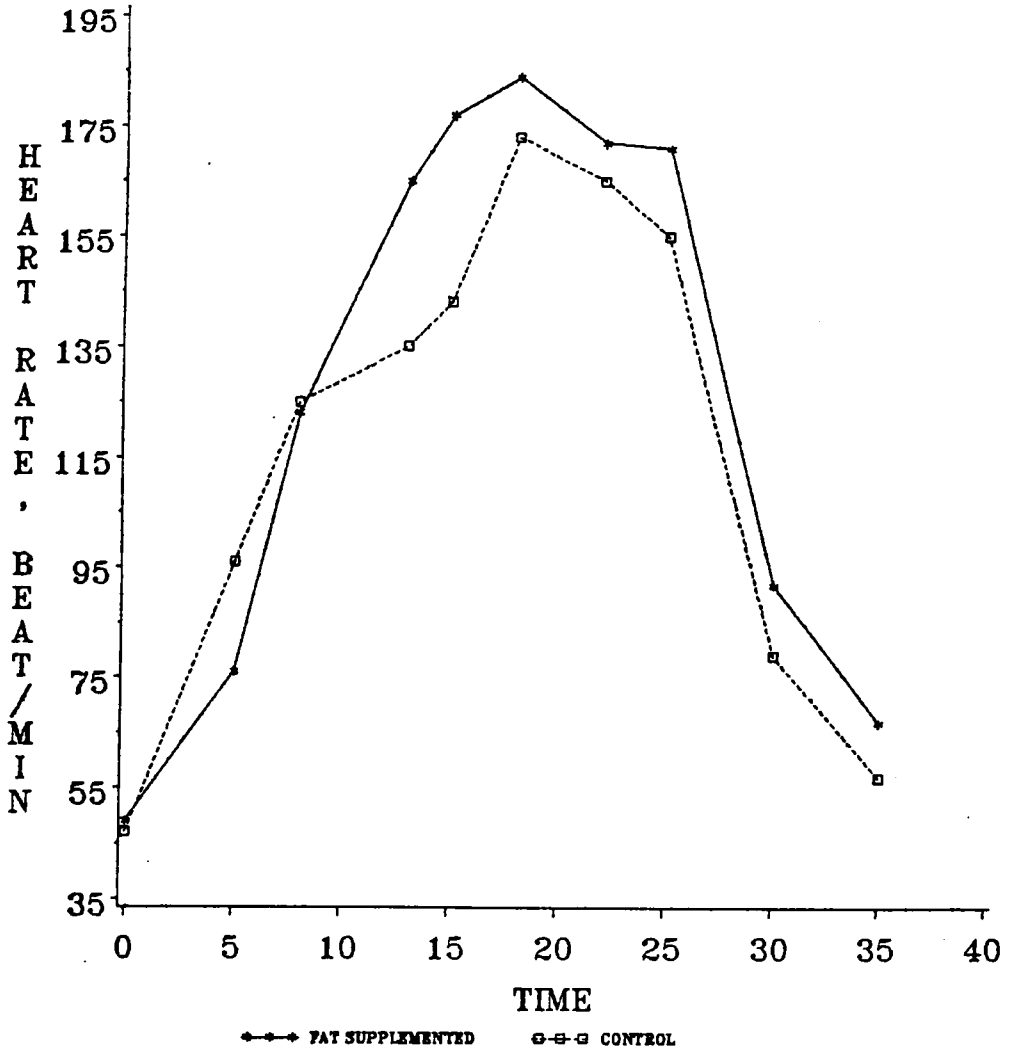

Figure 3 Heart Rate, Control and Fat-fed Horses. Experiment 2, SET 1, Pre-conditioning.

TABLE 8. Effect of Diet and Conditioning on Heart Rate. Experiment 2.

SET 1				SET 2			
		Diet				Diet	
Time of test	Speed	Control	Added fat	Time of test	Speed	Control	Added fat
min	m/min	---beat/min---		min	m/min	----beat/min---	
0	0	47	49	0	0	42	35
5	81	96	76	5	81	86	82
8	162	125	123	8	162	125	136
13	162	135	165	13	174	125	128
15	174	143	177	15	187	135	133
18	174	173	184	18	187	140	145
22	187	165	172	22	187	145	144
25	187/0	155/	171/	25	187/0	133/98	153/101
30	0	79	92	30	0	87	81
35	0	57	67	35	0	67	67

At walk the mean rates were 96 beats/min (control) and 76 beats/min (added fat)($P<.1$), which were also within normal rates for unconditioned horses although 96 is very close to the upper end of the range quoted by Snow (1983). At trot, mean rates were within the range 100/210 considered to be normal for unconditioned horses at this pace (Persson, 1983). The highest individual heart rate recorded for the SET was 196 beats/min which is below the 210 beats/min, above which heart rate ceases to be linear with respect to workload expressed as velocity (Persson, 1983).

A heart rate of 250 beats/min is considered to be the maximum normal rate for horses running on a treadmill (Persson, 1983). After the SET, all horses, except one, recovered to within 80% of their heart rate at walk, within 10 mins. Thus, except for one horse the workout could be considered to be submaximal and well within the fitness capacity of the horses. Individual results by horse are shown in table 16, appendix 2.

Blood Glucose. Overall, the horses fed the fat-supplemented diet showed a trend towards higher blood glucose levels (figure 4, table 9). The horses fed the high-fat diet tended to have higher blood glucose levels initially (97 vs 84 mg/dl), at 5 min (91 vs 72 mg/dl), 15 min (105 vs 77 mg/dl), 22 min (99 vs 97 mg/dl),25 min (99 vs 95 mg/dl), 5 min recovery (92 vs 81 mg/dl) and 10 min recovery (98 vs 87 mg/dl). There was no difference between the two groups at 13 min (95 vs 93 mg/dl) and the control group tended to have higher blood glucose at 8 min (85 vs 81 mg/dl) and at 18 min (85 vs 82 mg/dl).

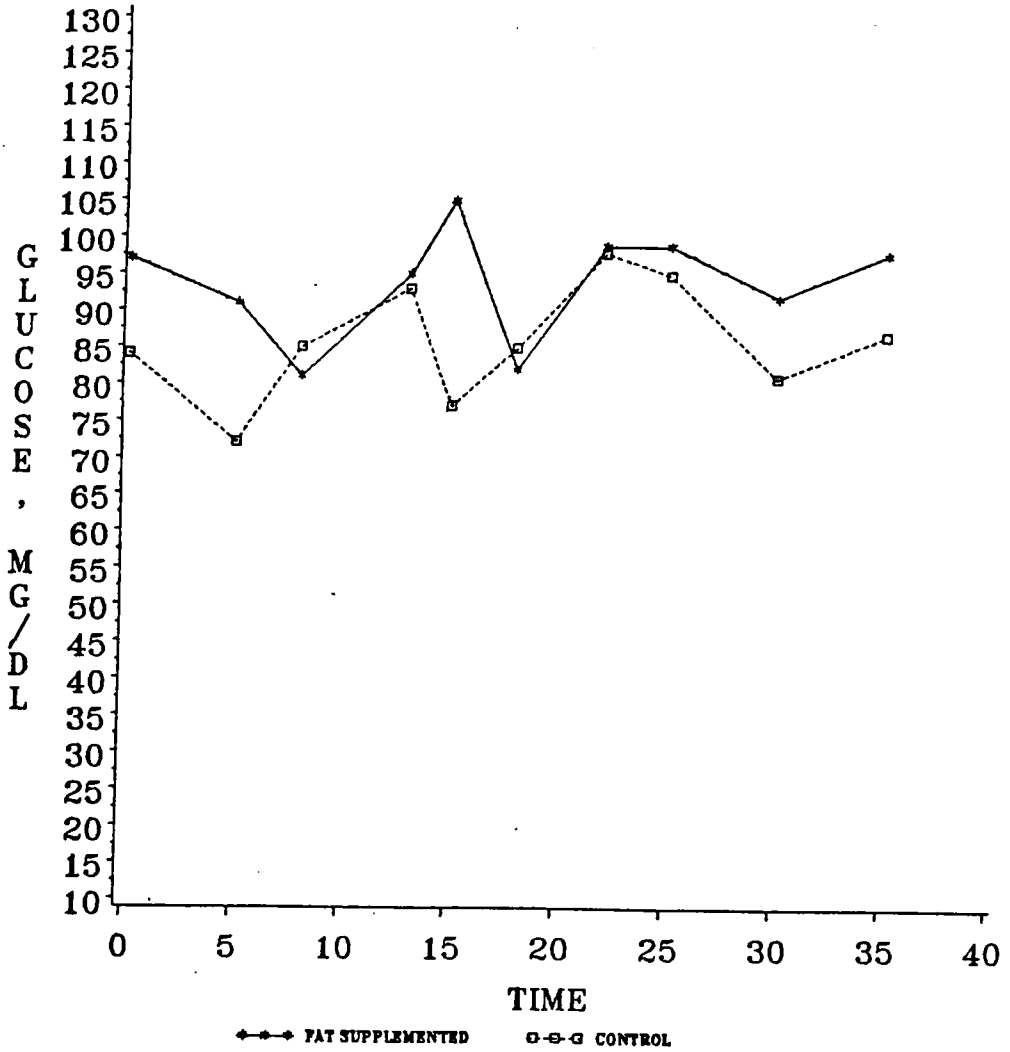

Figure 4. Blood Glucose Concentrations, Control and Fat-fed Horses, Experiment 2, SET 1, Pre-conditioning.

TABLE 9. Physiological Measurements in Response to Feeding Added Fat, SET 1 (Pre-Conditioning).

		Glucose by diet		Lactate by diet		CPK[a] by diet	
TIME	Speed	Control	Added fat	Control	Added fat	Control	Added fat
min	m/min	-- mg/dl--		-mmol/liter-		-- S.U.[b]--	
0	0	84	97	.63	.76	5	4
5	81	72	91	.75	.85	5	4
8	162	85	81	1.60	1.82	7	9
13	162	93	95	1.80	2.25	8	10
15	174	77	105	2.02	2.07	8	10
18	174	85	82	2.17	2.15	12	17
22	187	98	99	2.95	2.85	10	15
25	187/0	95	99	2.42	2.95	14	13
30	0	81	92	1.66	2.18	9	16
35	0	87	98	1.50	2.12	13	13

a Creatine phosphokinase
b Sigma units

Resting glucose levels were within the normal range for equine (Blackmore and Brobst, 1981).

There was no significant change in blood glucose during the exercise test which is in contrast to the results of Snow and MacKensie (1977) and Snow et al. (1982), who reported increases in blood glucose with exercise. However the exercise levels used by these workers were higher than the work load used in this trial. The blood glucose levels found in this trial are in agreement with those reported by Judson (1983) for submaximal work loads, and by Grosskopf and Van Rensburg (1983) for endurance horses at distances of 30 km and less. Individual results by horse are shown in table 17, appendix 2.

Blood Lactate The fat fed horses tended to have higher blood lactate levels (figure 5 and table 9). This group showed a trend towards higher lactate initially (.76 vs .63 mmol/liter), 5 min (.85 vs .75 mmol/liter), 8 min (1.82 vs 1.6 mmol/liter), 13 min (2.25 vs 1.8 mmol/liter), 15 min (2.07 vs 2.02 mmol/liter) 25 min (2.95 vs 2.4 mmol/liter), 5 min recovery (2.18 vs 1.66 mmol/liter) and 10 min recovery (2.12 vs 1.5 mmol/liter).

The control horses tended to have slightly higher blood lactates at 18 min (2.15 vs 2.17 mmol/liter) and 22 min (2.85 vs 2.9 mmol/liter). Blood lactate is exponetially related to both exercise heart rate and work load expressed as velocity (Englehart. 1973; Persson and Ullberg, 1974; Persson. 1983). In the first (preconditioning) SET, blood lactate was correlated with heart rate (r = .62).

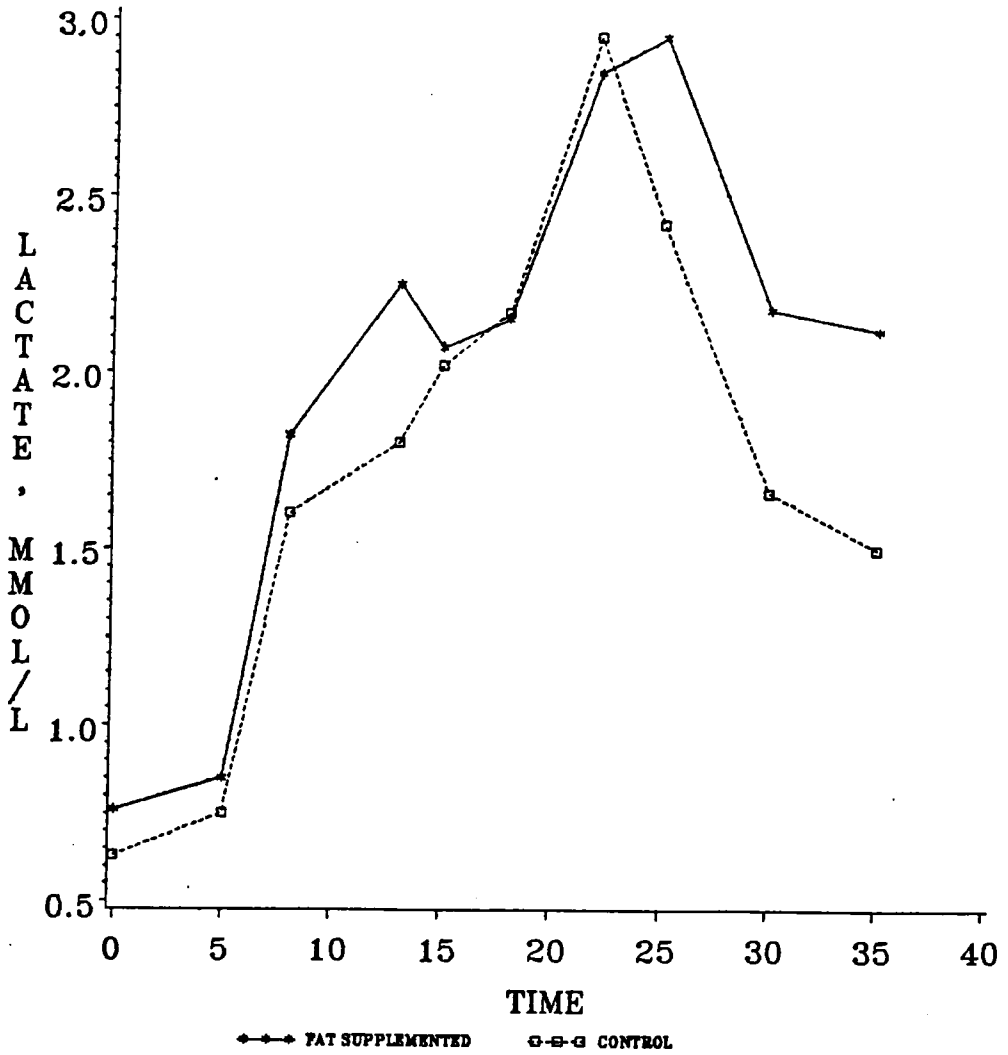

Figure 5 Blood Lactate Concentrations, Control and Fat-fed Horses. Experiment 2, SET 1, Pre-conditioning.

Blood lactate levels in excess of 4 mmol/liter are considered to indicate onset of blood lactate accumulation, sometimes called anaerobic threshold (Persson, 1983). No horses showed blood lactate levels in excess of 4 mmol/liter. Individual results by horse are shown in table 18, appendix 2.

Creatine Phosphokinase. There was no difference in creatine phosphokinase (CPK) levels between the the fat-fed horses and controls during the trial (figure 6 and table 9). There was no difference in initial, 5 min (on treadmill) and 10 min recovery (CPK) values between the group fed the diet containing added fat and controls, 4 vs 5 Sigma units (SU), and (13 vs 13 SU) respectivly. The horses fed the diet containing fat tended to have higher CPK values at 8 min (9 vs 7 SU), 13 min (10 vs 8 SU), 15 min (10 vs 7 SU), 18 min (17 vs 12 SU), 22 min (15 vs 10 SU) and 30 min (5 min recovery) (16 vs 9 SU). The control horses had slightly higher CPK values at 25 min (13 vs 14 SU). These values are within the normal range for equine (Blackmore and Brobst, 1980). This enzyme is usually measured as an indicator of muscle damage.

Activity in serum can rise with unaccustomed exercise or following more severe exercise during the latter part of training. Exceedingly high levels (up to 20,000 SU) are associated with severe muscle damage, e.g; rhabdomyolysis or myocardial infarction. The levels found in this trial were very low, indicating that the there was no muscle damage encountered during the exercise test. Individual results by horse are shown in table 19, appendix 2.

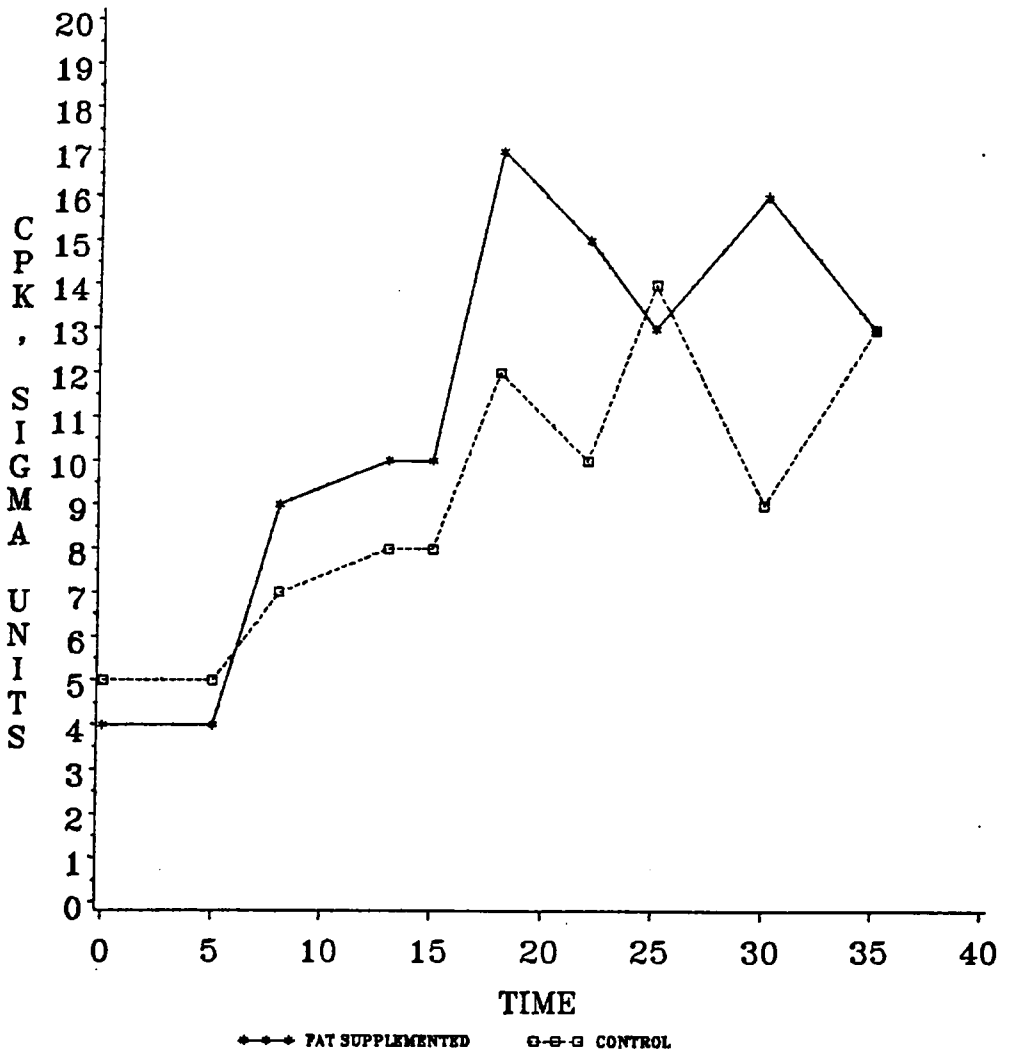

Figure 6, Creatine Phosphokinase Concentrations, Control and Fat-fed Horses. Experiment 2, SET 1, Pre-conditioning.

Standard Exercise Test 2, Post-Conditioning. There was no difference in initial temperatures between the two groups of horses (table 10). Post exercise temperatures were higher than initial or resting temperatures for all horses and were similar for the horses fed the control diet and the diet containing added fat. Individual results by horse are shown in table 20, appendix 2.

Horses fed a diet containing fat tended to have lower respiration rates initially (39 vs 37 breaths/min), but tended to have higher rates immediately post exercise (127 vs 141 breaths/min) and 5 mins post exercise (85 vs 97). The fat supplemented horses tended to recover more slowly than controls (84% vs 91% at 5 min post exercise) (table 10). None of the horses had inverted respiration/heart rates. Respiratory rates for all horses were higher than for the equivalent stage in SET 1. Respiration rate can be affected by several factors of which the exercise/work load is only one. Another important factor is environmental temperature and humidity. High ambient temperatures in conjunction with high humidity significantly compromises the horse's ability to lose heat and will cause an increase in respiration rate (Rose et al., 1983). The high respiration rates found in this SET could be a reflection of the higher ambient temperatures and humidity during this exercise test (29 C, 80% humidity during SET 2 compared with 15 C and 20% humidity during SET 1). Individual results by horse are shown in table 20, appendix 2.

TABLE 10.Post Conditioning Temperatures and Respiration Rates of Horses. Experiment 2, SET 2

	Diet			
	Control	Added fat	Control	Added fat
	---temp,C---		breaths/min	
Pre-exercise	37.0	37.0	39	37
Post-exercise	39.5	39.0	127	141
5 min post-exercise			85	97

Heart Rate. The horses fed the diet containing fat tended to have a lower initial heart rate (35 vs 42 beats/min) and at 5 min (82 vs 86 beats/min), 15 min (133 vs 135 beats/min), 22 min (144 vs 145 beats/min) and at 5 min recovery (81 vs 87 beats/min) (figure 7 and table 8). The horses fed the control diet tended to have lower heart rates at 8 min (136 vs 125 beats/min), 13 min (125 vs 128 beats/min), 18 min (145 vs 140 beats/min), 25 min (153 vs 132 beats/min), 26 min (1 to 2 min after getting off the treadmill) (101 vs 98 beats/min). There was no difference between the two groups at 10 min recovery (67 vs 67). Overall, heart rates remained below 160 beats/min despite the faster treadmill speeds.

Although a direct comparison between the pre- and post-conditioning SET cannot be made since the treadmill speeds used in the second SET were higher, resting and recovery rates can be compared. Conditioned horses showed lower heart rates at rest ($P<.05$) and had a tendency towards faster recovery rates. Individual results by horse are shown in table 20, appendix 2.

Blood Glucose. Horses fed a diet containing added fat tended to have lower blood glucose levels initially (94 vs 119 mg/dl), 8 min (72 vs 86 mg/dl), 13 min (71 vs 81 mg/dl), 15 min (76 vs 86 mg/dl), 18 min (83 vs 94 mg/dl), 25 min (98 vs 109 mg/dl), 30 min (5 min recovery) (128 vs 132 mg/dl) and 35 min (10 min recovery (100 vs 102 mg/dl), compared to controls (figure 8 and table 11).

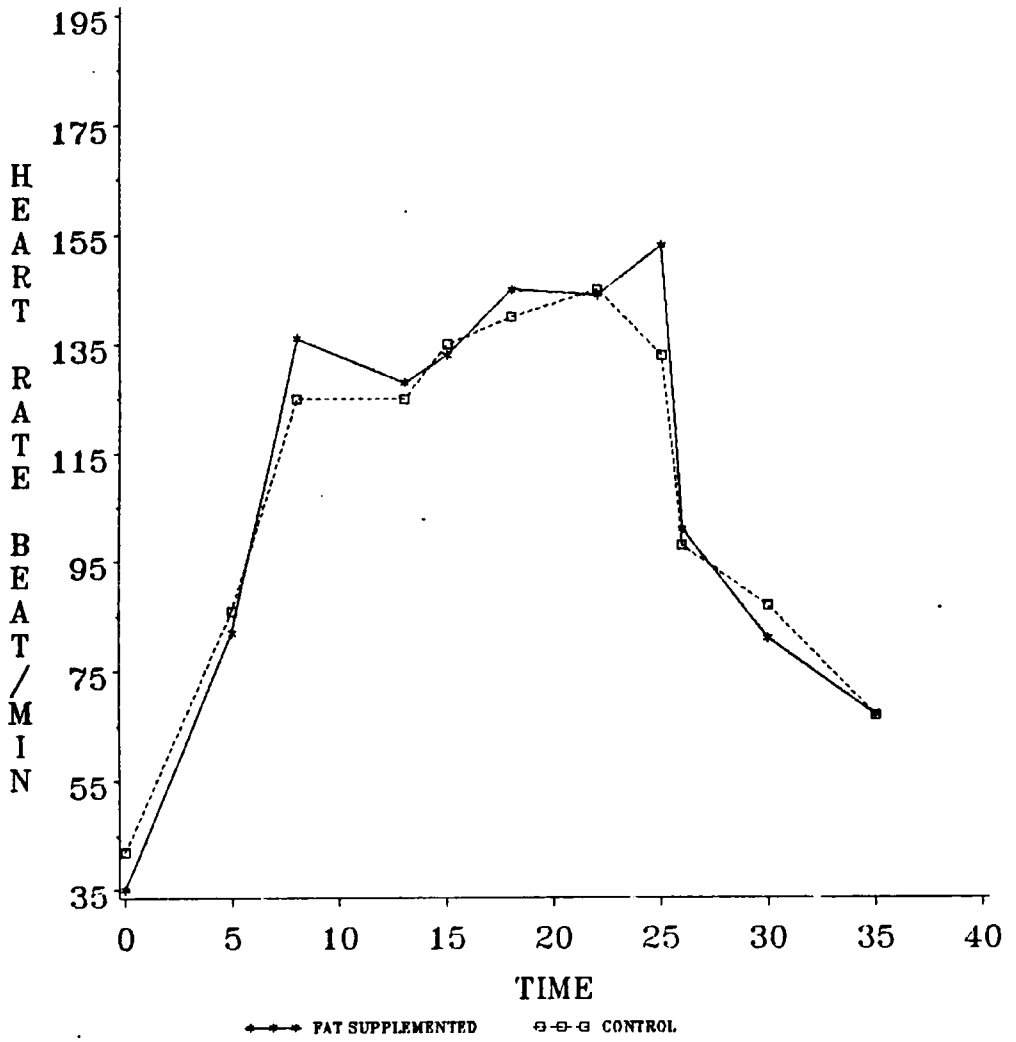

Figure 7. Heart Rate, Control and Fat-fed Horses. Experiment SET 2, Post-conditioning.

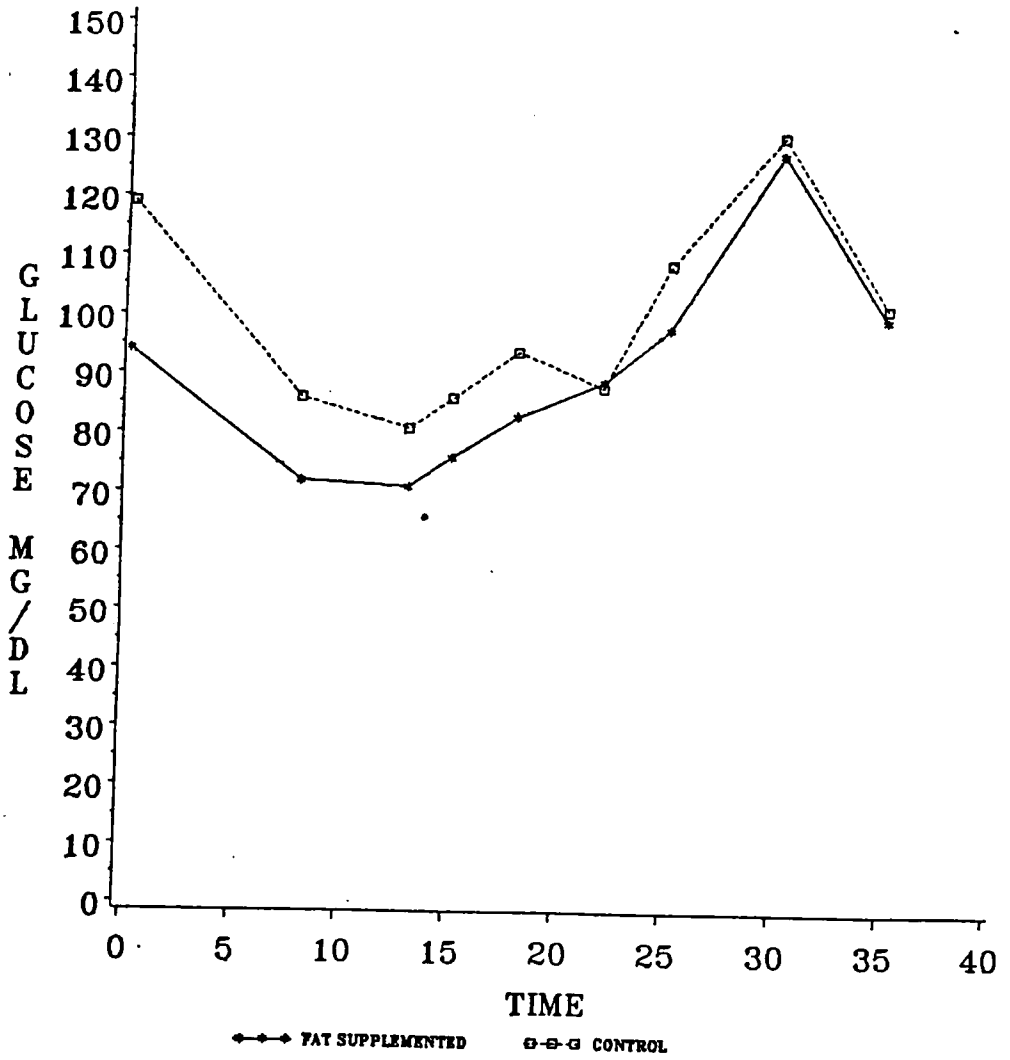

Figure 8. Blood Glucose Concentrations, Control and Fat-fed Horses. Experiment 2, SET 2, Post-conditioning.

TABLE 11. Blood Components in Response to Feeding Fat Post-Conditioning.

Time	Speed	Glucose by Diet		Lactate by Diet		BUN[a] by Diet		CPK[b] by Diet	
		Control	Added fat	Control	Added fat	Control	Added fat	Control	Added fat
min	m/min	---mg/dl---		--mmol/l--		---mg/dl---		--S.U.[c]--	
0	0	119.4	93.8	.43	.6	4.46	5.18	16	14
8	81	86.4	72.3	1.03	1.1	4.55	5.73	18	18
13	162	81.1	71.4	1.03	1.1	5.06	5.34	16	17
15	174	85.7	75.9	1.08	1.2	5.18	5.45	14	16
18	187	94.0	83.3	1.5	1.7	5.00	5.44	21	13
22	187	88.4	89.2	1.65	2.2	5.39	5.41	19	14
25	187	109.3	98.2	1.92	2.5	4.64	5.41	14	12
30	0	131.8	128.4	1.92	2.3	4.27	4.85	13	12
35	0	101.8	99.8	1.55	2.5	3.52	4.42	13	9

[a] Blood urea nitrogen
[b] Creatine phosphokinase
[c] Sigma units

There was no difference between the two groups at 22 min. As mentioned above, usually blood glucose levels rise with exercise (Blackmore and Brobst, 1981), but in this SET, blood glucose levels fell until 25 min (end of exercise) then rose, temporarily for the control group, and remained above control levels for the fat-fed group. This may have been due to the short duration of the exercise test and the submaximal level of the workload. Snow et al. (1983) suggested that there is a lag time in the response of blood glucose levels to onset and cessation of exercising. Individual results by horse are shown in table 21, appendix 2.

Blood Lactate. Fat-supplemented horses showed a trend towards higher blood lactate levels initially (.6 vs .425 mmol/liter), and throughout the SET, with the greatest difference being at 22 min (2.2 vs 1.65 mmol/liter), 25 min (2.5 vs 1.61 mmol/liter), 5 min recovery (2.3 vs 1.92 mmol/liter) and 10 min (2.5 vs 1.55 mmol/liter). There was no difference between groups at 8 min (1.1 vs 1.03 mmol/liter), 13 min (1.1 vs 1.03 mmol/liter), 15 min (1.2 vs 1.08 mmol/liter) and 18 min (1.7 vs 1.5 mmol/liter) (table 11 and figure 9). These findings are in contrast to those reported by Webb et al. (1987) who found lower blood lactate levels after a workout in horses fed a diet containing additional fat. The discrepancy in results may be due to the fact that Webb et al. (1987) added corn oil to a traditional diet of hay and corn, whereas in this study, the diets were isocaloric, with no readily digestible carbohydrate source being fed to the horses on the high-fat diet.

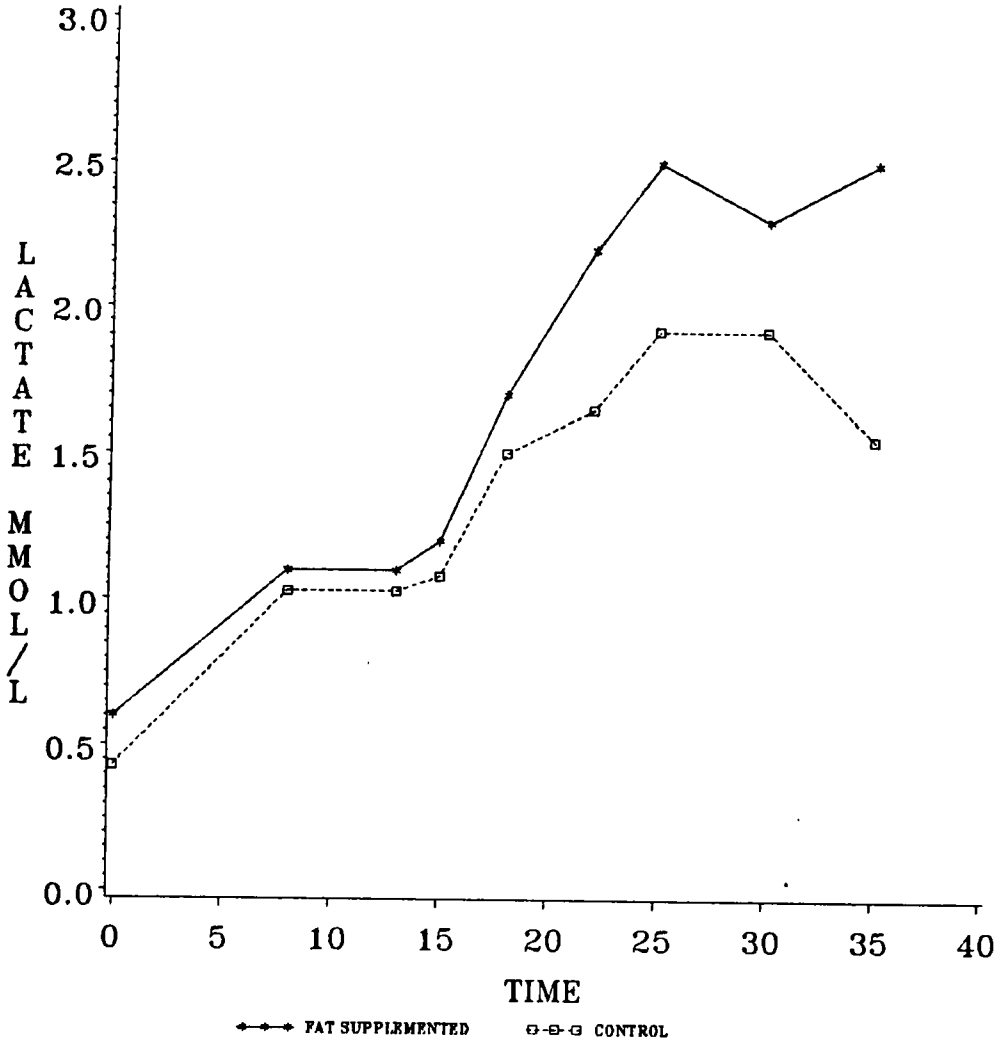

Figure 9. Blood Lactate Concentrations, Control and Fat-fed Horses, Experiment 2, SET 2, Post-conditioning.

The horses in this study may have been marginally deficient in soluble carbohydrate and thus may have been supplying gluconeogenic precursors to the liver via glycolysis, hence higher levels of blood lactate.

No horse had a blood lactate level of 4 mmol/liter or higher at any time during the test. There was no correlation between blood lactate levels and either heart rate or velocity in the second SET, which may be a reflection of the increased fitness of the horses, the speeds and duration of the second SET being insufficient to adequately test animals trained to this level of fitness. Individual results by horse are shown in table 22, appendix 2.

Blood Urea Nitrogen (BUN). Initial values for BUN tended to be higher in fat supplemented horses (5.18 vs 4.46 mg/dl) and remained higher throughout the SET (table 11 and figure 10). The reason for the higher BUN levels in the horses fed fat is not obvious. In other studies on feeding fat, BUN has not been measured, so there are no other studies to compare the results to. It is possible that if the horses on the high-fat diet were carbohydrate deficient, that in addition to using glycolysis to supply gluconeogenic precursors for the liver, they could also use the alanine cycle (as is found in starvation, McGilvery, 1983). Thus there would be increase in urea from the liver as the alanine is deaminated and the pyruvate produced can be used for glucose production (McGilvery 1983). Individual results by horse are shown in table 23, appendix 2.

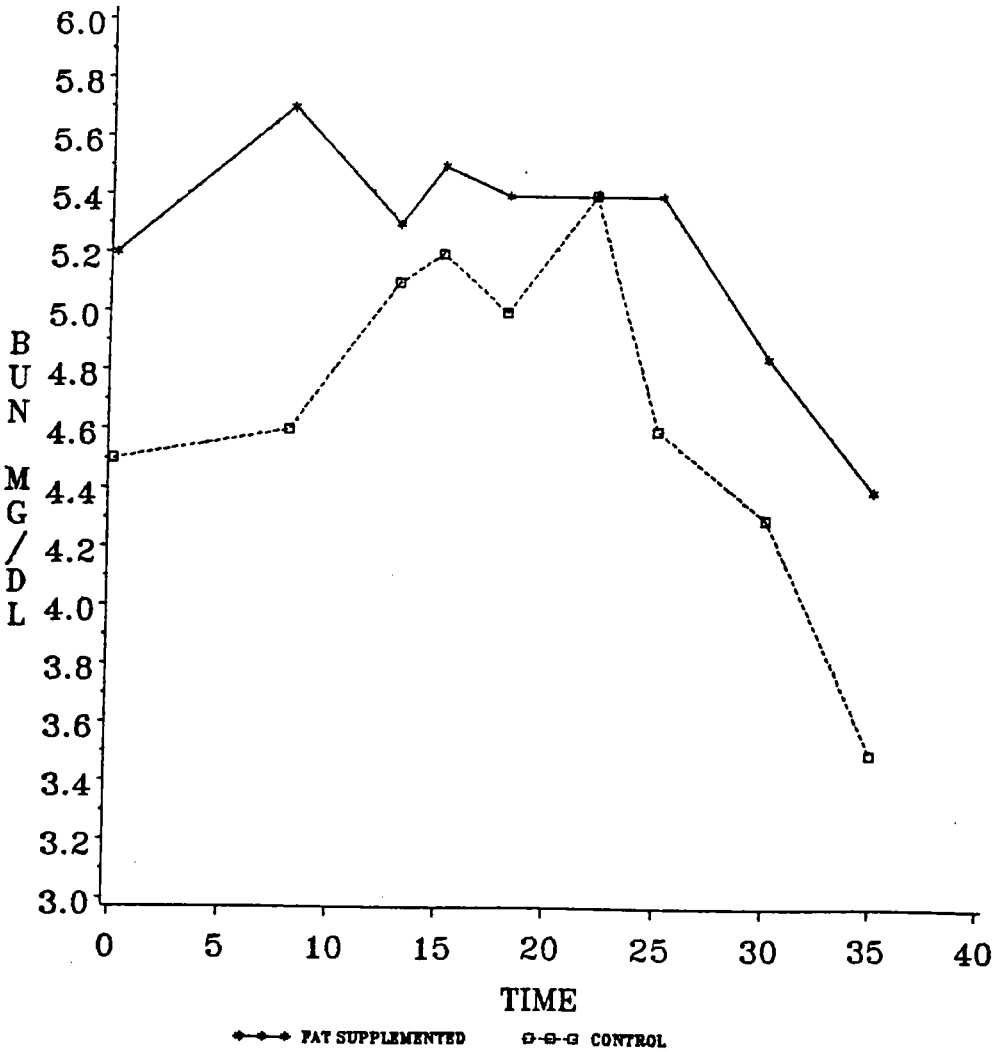

Figure 10, Blood Urea Nitrogen Concentrations Control and Fat-fed Horses. Experiment 2,SET 2, Post-conditioning.

Creatine Phosphokinase. Values for pre-exercise, 8 min, 13 min and 15 min for both groups of horses were much higher than during the first SET (14 and 16 SU vs 4 and 5 SU), (18 and 18 SU vs 9 and 8 SU), (17 and 16 SU vs 10 and 8 SU) and (16 and 14 SU vs 10 and 7 SU), respectivly, (table 11 and figure 11). There was no difference between the fat-supplemented and control horses, however. At 18 and 22 min the control horses tended to have higher CPK levels (13 vs 21 SU) and (14 vs 19 SU), respectivly. At 25 min and during recovery there was no difference between the two groups. The elevated pre-exercise levels may reflect the more strenous workouts that the horses had done during the latter part of training (Blackmore and Brobst 1980). That there was no difference between the groups indicates that it was a training rather than a diet effect. Individual results by horse are shown in table 24, appendix 2.

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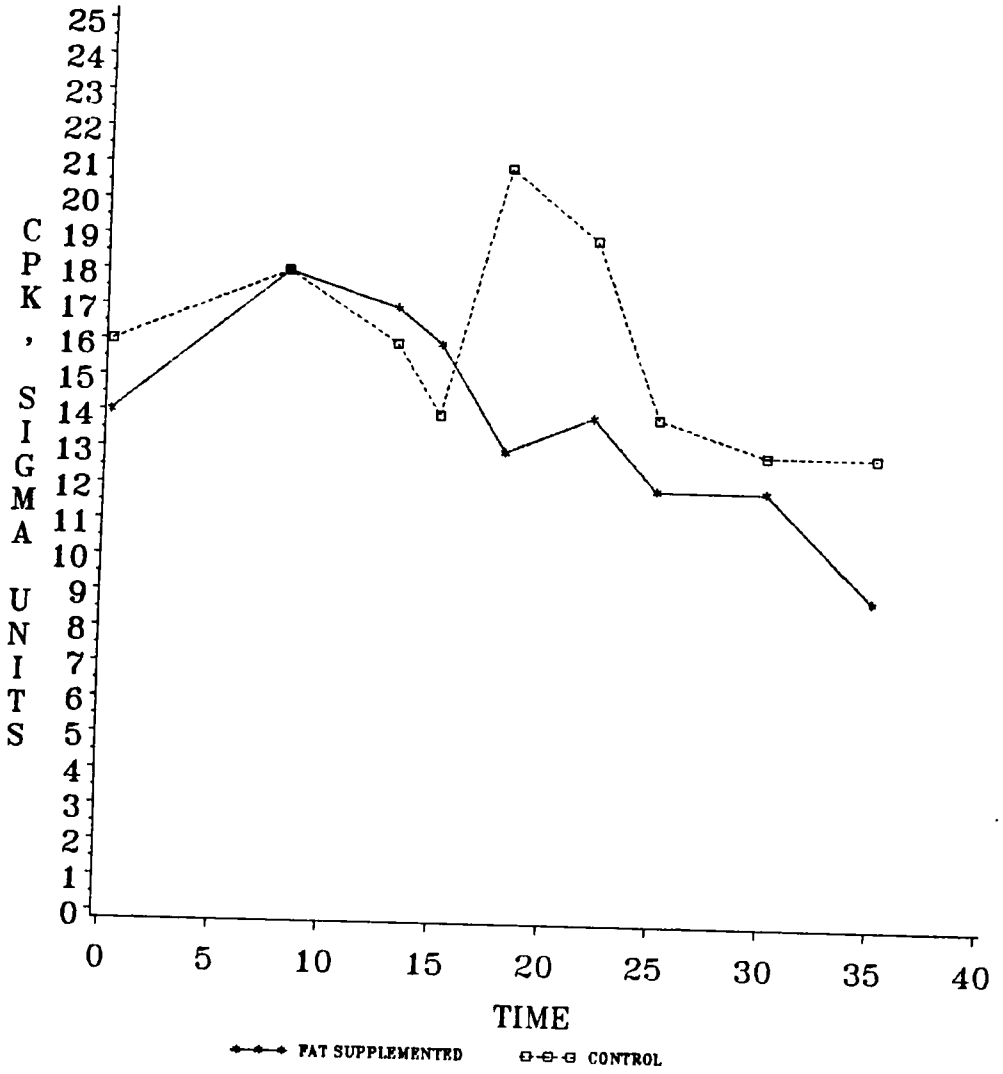

Figure 11. Creatine Phosphokinase Concentrations, Control and Fat-fed Horses. Experiment 2, SET 2, Post-conditioning.

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Conclusions

A moderate level of exercise appears to enhance digestion in the equine, which is in contrast to expected results. However, the exercise program used in this study did not include very much fast work (in excess of 550 m/min), for various reasons. Therefore, the study did not indicate whether this effect would be the same at higher levels of exercise. Most competition horses are trained considerably harder than used in this study. Horses in training for racing, polo, cutting and 3-d eventing, are expected to perform at fairly high speeds (well in excess of 550 m/min), so for these activities these results may not have much relevance. Endurance and dressage horses, however, often work within these speeds, albeit for somewhat longer periods, so the results may be pertinent for them.

On a practical basis there might be some benefit here for the producer of young horses intended for sport, a mild level of exercise could help with digestion of feed as well as the appearance of the young stock. Further work is needed to establish the extent to which exercise is beneficial, and what level of exercise produces the best results. Also, it would be useful to find out which type of exercise (aerobic vs anaerobic or a combination of both) would be of most benefit.

It might also be interesting to investigate whether there were any breed differences in response to exercise, in this study a mixed

group (with respect to breed)were used, thus, any breed differences were impossible to identify.

Supplying part of the dietary calories as fat appears to be viable in the equine. There were no deleterious effects at this moderate level of exercise stress, but it might be preferable to supply the fat as an addition to a standard carbohydrate diet. The horses on this diet might have been carbohydrate deficient, as evidenced by the falling levels of blood glucose despite the short duration and the aerobic nature of the exercise test used. Also of interest is the rising BUN levels which would seem to indicate an involvement of protein, which has not previously been shown in the horse.

The essentiality of carbohydrate has never been shown in the equine diet. It may be that at this and higher levels of exercise essentiality of a dietary carbohydrate source could be shown. Webb et al (1987) fed fat in addition to a standard diet and found that their horses could not maintain glucose homeostasis. They concluded that their animals were possibly glycogen deficient. It is possible that if the horses fed fat in this study were worked harder (anaerobically) or for a longer period of time they also might have shown an inability to maintain glucose homeostasis.

This study did not stress the horses sufficiently during the exercise tests as evidenced by the low blood lactate levels shown and the low heart rates as compared with those seen in the literature, That there was a correlation between blood lactate and time and between heart rate and time only during the pre-conditiong SET indicate

that the post-conditioning SET produced insufficient stress. A treadmill which can run at faster speeds or running the test for a longer period of time would be nessecary. To elucidate further the effect of exercise and dietary fat on muscle energy metabolisim it would be useful to measure RQ under various work loads and at different levels of fitness on diets with different fat contents. Further work would be interesting on the effects of various exercise levels and high-carbohydrate diets on the BUN and the utilization of body protein. Of particular interest is the interaction between dietary fat and conditioning on the apparent digestibility of energy, this effect warrants further investigation.

Choice white grease might not be the ideal fat of choice for equines, since it took a while for the horses to accept the fat used in this study. Horses at very high levels of work are notoriously picky eaters, and these animals concievably have the greatest need for additional energy in their diet. Thus, to add an unpalatable ingredient to their ration would be self-defeating. A much more palatable source of fat is corn or peanut oil on a dextrose base, and this would presumably be a more advantageous fat source for equines.

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Appendix 1

TABLE 1 INDIVIDUAL DATA FOR HORSES USED IN EXPERIMENT 1.

Name	No	Breed	Age	Weight	Treatment
			yrs	kg	
Robber	1	SB[a]	4	440	Control
Rebel	2	SB	4	460	Exercise
Roger	3	SB	4	405	Exercise
Brigand	4	SB	4	410	Control
Monty	5	Morg[b]	7	567	Exercise
Sultan	6	Ar/QH[c]	6	525	Control
Domino	7	TB[d]	7	493	Control
Slim	8	SB	6	517	Exercise

[a] American Saddlebred
[b] Morgan
[c] Arabian/Quarter horse cross
[d] Thoroughbred

Appendix 1

TABLE 2 DAILY DRY MATTER INTAKE OF HORSES.EXPERIMENT 1.

	Horse							
Week	1	2	3	4	5	6	7	8
	kg							
1 and 2	9	9	8	8	11	10.5	10	10
3 and 4	9	9	8	8	11	10.5	10	10
5 and 6	8	8.5	8	8	10	10	9.5	9
6 and 7	8	8.5	8	8	9	10	9.5	9
8 and 9	8	9	8	8	9	10	9.5	9

Appendix 1

TABLE 3. INDIVIDUAL WEIGHTS OF HORSES TRIAL 1.

Horse no	Initial wt	Week 2	4	6	8	Gain
	kg					
1	440	448	457	475	480	+40
2	460	470	479	475	480	+20
3	405	407	425	425	430	+25
4	410	418	427	475	450	+40
5	567	557	564	560	560	-7
6	525	527	529	560	550	+25
7	493	490	509	525	520	+27
8	517	507	525	500	500	-17

Appendix 1

TABLE 4 DAILY EXERCISE REGIME OF HORSES. EXPERIMENT 1.

Week	Time at paces			
	Walk	Trot	Canter	Gallop
	----------mins----------			
1	20-30			
2	35-40	5-15		
3	40	15-20		
4	40	20-30		
5	40	35-40	3	
6	40	40	5,3[a]	
7	40	40	5,5	
8	40	40	5,3	1,2[b]
9	40	40	5,3	2,3

[a] Two separate canters with 3 min of walk between
[b] Two separate gallops with 5 min of walk between

Appendix 1

TABLE 5 . MEAN RESTING HEARTRATES FOR HORSES

Horse no	Week 1	2	3	4	5	6	7	8	9	10
	----------beat/min----------									
1	45	44	50	40	44	45	46	44	46	45
2	40	36	38	36	34	37	36	34	36	35
3	42	40	39	39	36	37	36	44	40	45
4	40	40	35	36	37	40	38	39	40	46
5	40	39	45	44	37	36	35	35	39	36
6	40	40	43	39	40	36	38	37	40	34
7	39	43	43	40	38	37	40	39	35	39
8	42	40	39	50	39	48	45	39	36	34

Appendix 1

TABLE 6. PROTOCOL FOR TRIAL 1, SET 1.

Time (mins)	Speed (m/min)	Heart rate measured	Blood sample taken
0	0	*	*
3	67	*	
5	161	*	
10	161	*	
15	174	*	
17	188/stop	*	*
22	0 rest	*	*
27	0 rest	*	*

Appendix 1

TABLE 7. HEARTRATES BY HORSE, EXPERIMENT 1.

Pace	Time	Speed	Horse no by treatment: Control 1	Control 4	Control 6	Control $\bar{X}$[a]	Conditioned 2	Conditioned 3	Conditioned 8	Conditioned $\bar{X}$[a]
	min	m/min	beats/min							
Rest	0	0[f]	45	46	33	41	35	45	33	37
Walk	3	67[b]	89	116	85	96	88	90	99	92
Trot	5	161[c]	205	200	173	193	162	160	183	168
Trot	10	161	195	189	193	192	159	149	223	177
Trot	15	174[d]	203	196	200	199	169	166	210	181
Trot	17	188[e]	123	145	136	134	103	112	111	108
Results	22	0[f]	76	95	76	82	54	52	64	57
Results	27	0[f]	62	68	64	64	48	45	53	48

[a] Means differ (P<.05)
[b] Walk
[c] Slow trot
[d], [e] Faster trot
[f] Rest or recovery

Appendix 1

TABLE 8. BLOOD LACTATE AND PACKED CELL VOLUMES TRIAL 1 SET 1.

Horse no	Treatment	Lactate by time, min				Packed cell volume by time, min			
		0	17	22	27	0	17	22	27
		-------mmol/liter----				---------%----------			
1	Control	1.3	6.8	5.5	4.8	37	48	44	40
4	Control	1.5	5.7	4.3	3.7	31	37	37	31
6	Control	1.1	3.6	3.0	2.8	41	47	44	44
	Avg	1.3	5.4	4.3	3.8[a]	36	44	41	38
2	Exercise	1.1	1.6	1.3	1.4	31	52	38	41
3	Exercise	1.1	1.0	1.0	1.0	28	47	43	41
8	Exercise	1.1	2.2	2.7	2.0	37	40	40	37
	Avg	1.1	1.6	1.5	1.5[a]	32	46	40	40

[a]Means differ ($P<.05$)

Appendix 1

TABLE 9. APPARENT DIGESTIBILITY OF ENERGY, PROTEIN AND CELL WALL FRACTIONS BY INDIVIDUAL HORSES. EXPERIMENT 1.

Horse no	Treatment	Component						
		Dry matter	Crude protein	Cell contents	NDF	ADF	Cellulose	Energy
		%						
1	Control	62.02	61.96	77.25	60.93	30.65	32.89	61.60
4	Control	59.41	63.59	78.81	56.14	21.68	24.39	58.85
6	Control	60.82	65.02	75.63	60.12	23.42	30.95	59.24
7	Control	56.17	62.25	73.75	54.97	14.84	24.63	56.41
	Avg[c]	59.61[a]	63.21[b]	76.36	58.04	22.65[a]	28.21	59.02
2	Exercise	66.61	71.19	79.87	65.61	41.46	43.00	65.76
3	Exercise	65.11	70.09	78.64	63.96	39.16	41.42	65.02
5	Exercise	67.70	70.37	79.49	67.65	45.56	49.26	68.02
8	Exercise	58.19	63.48	76.79	55.24	18.15	20.02	57.99
	Avg[c]	64.40[a]	68.78[b]	78.69	63.12	36.08[a]	38.42	64.20

[a] Means differ (P<.1)
[b] Means differ (P<.05).
[c] Mean for each treatment (N = 4)

APPENDIX 2

Appendix 2

TABLE 10. INDIVIDUAL DATA FOR HORSES USED IN EXPERIMENT 2.

Horse name	No	Breed	Age	Weight	Treatment
			yr	kg	
Roger	1	SB[a]	4	385	control
Robber	2	SB[a]	4	408	fat
Points	3	SB[a]	4	408	control
Brigand	4	SB[a]	4	417	fat
Trapper	5	QH/TB[b]	6	489	control
Beau	6	QH/TB[b]	4	430	fat
Monty	7	Morg[c]	7	528	control
Slim	8	SB[a]	7	478	fat

a American Saddlebred
b Quarter horse/Thoroughbred
c Morgan

Appendix 2

TABLE 11 .DAILY DRY MATTER INTAKE OF HORSES. EXPERIMENT 2.

	Horses							
Week	1	2	3	4	5	6	7	8
	kg							
1 and 2	9	9	8	8	11	10.5	10	10
3 and 4	9	9	8	8	11	10.5	10	10
5 and 6	8	8.5	8	8	10	10	9.5	9
6 and 7	8	8.5	8	8	9	10	9.5	9
8 and 9	8	9	8	8	9	10	9.5	9

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TABLE 12. INDIVIDUAL WEIGHTS OF HORSES. EXPERIMENT 2.

Horse no	Initial wt	Week				Gain
		2	4	6	8	
	kg					
1	385	385	390	395	400	+15
3	411	418	425	415	436	+25
5	479	486	486	481	497	+18
7	520	525	522	525	532	+12
2	404	397	397	393	395	-13
6	430	436	434	443	448	+18
4	409	411	404	400	409	+8
S1	479	482	472	468	477	-1

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TABLE 13. PROTOCOL FOR STANDARD EXERCISE TESTS (SET). EXPERIMENT 2.

	SET 1[a]		SET 2[b]	
Time	Speed	Blood sample	Speed	Blood sample
min	m/min		m/min	
0	0	*	0	*
5	81	*	81	
8	162	*	174	*
13	174	*	187	*
15	174	*	187	*
18	187	*	187	*
22	187	*	187	*
25	187/0	*	187/0	*
30	0	*	0	*
35	0	*	0	*

[a] Pre-Conditioning
[b] Post-Conditioning

Appendix 2

TABLE 14. APPARENT DIGESTIBILITY OF ENERGY, CRUDE PROTEIN AND CELL WALL FRACTIONS BY INDIVIDUAL HORSES, PRE-CONDITIONING.

Treatment	Horse no	Component					
		Dry matter	Crude protein	Cell contents	NDF[a]	ADF[b]	Energy
		%					
Control	1	64.61	63.57	83.73	43.76	18.89	68.28
Control	3	66.94	57.25	82.20	50.32	29.11	65.70
Control	5	64.46	72.73	78.75	48.93	15.73	63.29
Control	7	73.12	72.91	83.35	61.99	34.82	65.38
Avg		67.28	66.61	82.01	51.30	24.64	65.66
Fat	2	57.96	68.91	76.38	31.22	18.41	87.91
Fat	6	54.29	61.78	74.72	24.72	11.55	88.86
Fat	4	55.58	61.01	72.90	30.43	13.42	90.30
Fat	8	58.79	67.34	78.26	30.52	27.95	89.75
Avg		56.65	64.76	75.57	29.2	17.83	89.20

[a] Neutral detergent fiber
[b] Acid detergent fiber

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TABLE 15. APPARENT DIGESTIBILITY OF ENERGY, CRUDE PROTEIN AND CELL WALL FRACTIONS BY INDIVIDUAL HORSES, POST-CONDITIONING.

Treatment	Horse no	Component					
		Dry matter	Crude protein	Cell contents	NDF[a]	ADF[b]	Energy
		----------%----------					
Control	1	72.46	73.12	85.87	55.78	35.33	66.67
Control	3	69.94	61.67	81.66	62.67	29.59	50.41
Control	5	63.90	61.24	80.81	39.89	14.85	45.51
Control	7	63.90	69.47	74.15	48.29	34.82	55.47
Avg		67.55	66.37	80.62	51.70	23.38	54.52
Fat	2	60.05	75.37	77.44	23.81	23.66	88.53
Fat	6	59.95	66.57	73.62	30.87	25.39	89.39
Fat	4	55.63	75.57	76.84	16.94	10.83	88.68
Fat	8	67.42	77.27	78.77	50.22	47.17	88.23
Avg		60.76	73.69	76.67	30.40	26.76	88.70

[a] Neutral detergent fiber
[b] Acid detergent fiber

Appendix 2

TABLE 16. INDIVIDUAL HEART RATES, RESPIRATION RATES, AND BODY TEMPERATURES EXPERIMENT 2, SET 1.

	Horse no. and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
	Rectal temperature, C									
0	28	28	28	28	28	27	27	27	28	27
30	30	29	29	29	29	30	29	29	29	29
Min	Heart rate, beats/min									
0	48	47	68	33	49	56	49	47	37	47
5	71	72	90	69	76	74	95	95	120	96
8	137	134	132	90	123	125	130	120	124	125
13	129	166	175	190	165	130	141	128	142	135
15	150	180	180	196	177	120	150	168	135	143
18	174	190	182	190	184	185	184	180	141	173
22	170	166	185	165	144	180	170	175	133	145
25	168	162	189	165	153	156	171	180	112	132
30	65	154	86	62	81	73	90	85	67	87
35	56	94	67	49	67	66	60	50	51	67
	Respiration, breaths/ min									
0	24	24	40	20	27	20	30	16	20	22
30	128	160	120	70	120	136	90	104	90	105
35	64	80	60	40	61	68	60	44	30	51

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TABLE 17. BLOOD GLUCOSE CONCENTRATIONS. EXPERIMENT 2, SET 1.

	Horse no and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	mg/dl									
0	88	130	75	96	97	95	70	59	111	84
5	136	54	96	80	91	88	81	106	99	72
8	93	63	89	78	81	100	98	81	63	85
13	96	47	55	82	95	120	72	88	93	93
15	88	51	189	90	105	82	76	74	77	77
18	80	49	118	82	82	89	82	85	85	85
22	94	63	136	103	99	94	79	79	138	97
25	95	58	137	103	98	102	102	82	95	95
30	89	75	116	89	92	87	78	80	81	81
35	91	96	123	81	98	119	70	77	83	87

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TABLE 18. BLOOD LACTATE CONCENTRATIONS. EXPERIMENT 2, SET 1.

	Horse no and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	mmol/liter									
0	.6	.65	1.3	.5	.76	.6	.6	.8	.6	.6
5	.8	.6	1.6	.5	.9	.8	.9	.7	.7	.8
8	1.9	.8	3.4	1.2	1.8	1.3	2.5	1.5	1.2	1.6
13	1.2	2.1	3.1	.8	2.25	1.9	2.5	2.1	1.8	1.8
15	1.5	3.0	2.7	1.0	2.07	2.0	2.2	2.0	1.9	2.02
18	2.1	2.9	2.4	1.2	2.15	2.0	2.9	2.3	1.5	2.17
22	2.2	3.8	3.6	1.8	2.85	3.8	2.8	3.2	1.8	2.9
25	2.8	3.9	3.0	2.1	2.95	2.4	2.7	3.3	1.5	2.4
30	1.9	3.5	2.4	1.0	2.18	1.6	1.7	1.9	1.4	1.66
35	2.5	2.5	2.3	1.2	2.12	1.6	1.4	1.8	1.2	1.5

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TABLE 19. CREATINE PHOSPHOKINASE CONCENTRATIONS. EXPERIMENT 2, SET 1.

	Horse no and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	Sigma units									
0	5	5	3	4	4.25	3	5	4	6	4.6
5	3	5	5	4	4.25	6	2	5	6	4.75
8	17	9	5	6	9.25	9	1	6	13	7.25
13	14	12	7	6	9.75	7	7	8	8	7.5
15	14	5	9	13	10.25	2	10	10	5	6.75
18	12	13	28	14	16.75	7	4	19	16	11.5
22	13	15	20	13	15.25	9	5	9	16	9.25
25	13	10	15	13	12.75	3	4	16	33	14.0
30	16	23	9	16	16.0	4	5	9	19	9.25
35	13	6	17	15	12.75	13	5	15	17	12.50

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TABLE 20. INDIVIDUAL HEART RATES, RESPIRATION RATES AND BODY TEMPERATURES. EXPERIMENT 2, SET 2.

	Horse no and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min										
	----------Rectal temperature, C----------									
0	28	28	28	27	28	28	28	28	28	28
30	31	29	31	30	30	30	29	30	30	30
	----------Heart rate, beats/min----------									
0	37	38	35	32	35	49	49	36	35	42
5	92	71	67	99	82	89	82	72	103	86
8	134	128	135	147	136	129	121	135	115	125
13	120	129	118	143	127	134	137	121	108	125
15	133	135	120	142	132	142	134	145	118	135
18	140	143	146	150	145	145	147	137	131	140
22	140	144	136	154	143	145	150	159	127	145
25	144	152	156	159	153	100	155	150	124	132
26	99	97	106	101	101	75	105	100	111	98
30	76	79	93	75	81	100	95	70	85	88
35	67	70	72	60	67	63	74	64	68	67
	----------Respiration, breaths/min----------									
0	44	40	48	24	39	46	24	44	36	38
30	125	80	144	160	127	120	155	140	150	141
35	90	68	100	80	85	90	100	120	78	97

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TABLE 21. BLOOD GLUCOSE CONCENTRATIONS. EXPERIMENT 2, SET 2.

	Horse no. and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	mg/dl									
0	90.8	90.4	85.5	108.7	93.9	102.7	110.8	177.7	86.5	119.4
8	91.2	53.5	77.6	67.0	72.3	95.4	89.7	85.0	75.5	86.4
13	100.0	62.7	73.4	49.6	71.4	97.3	73.3	81.1	72.8	81.1
15	100.0	61.9	89.2	52.7	75.9	101.9	77.0	88.8	75.0	85.6
18	94.0	71.2	106.6	61.7	83.3	103.1	88.1	92.3	92.3	93.9
22	110.4	69.2	106.6	70.7	89.2	89.6	90.8	88.4	85.0	88.5
25	114.6	87.7	109.8	80.7	98.2	101.5	148.3	104.6	82.8	109.3
30	114.6	113.5	184.2	101.3	128.4	109.6	107.1	108.8	101.8	106.8
35	118.8	75.4	121.9	83.4	99.9	113.5	102.4	104.2	87.1	101.8

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TABLE 22. BLOOD LACTATE CONCENTRATIONS. EXPERIMENT 2, SET 2.

	Horse no and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	mmol/liter									
0	.65	.6	.55	.65	.6	.45	.4	.45	.4	.43
8	.9	.8	1.8	.8	1.1	.8	1.35	1.25	.7	1.025
13	1.3	.8	1.3	1.0	1.1	.8	1.7	.95	.65	1.025
15	.85	.9	1.9	1.3	1.2	.9	1.6	1.15	.65	1.075
18	2.1	2.9	2.4	1.2	2.15	2.0	2.9	2.3	1.5	2.17
22	1.9	1.2	3.5	2.2	2.2	.95	2.3	2.5	.75	1.65
25	2.9	1.15	3.5	2.4	2.5	1.15	2.5	2.5	1.2	1.92
30	2.7	1.15	3.2	2.3	2.3	.9	1.5	2.6	1.2	1.92
35	2.7	1.1	3.9	2.3	2.5	.85	1.4	2.3	1.25	1.55

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TABLE 23. BLOOD UREA NITROGEN CONCENTRATION. EXPERIMENT 2, SET 2.

	Horse no. and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	mmol/liter									
0	7.9	4.7	5.1	2.94	5.18	3.96	5.08	4.28	4.5	4.46
8	4.45	5.97	5.35	7.14	5.73	3.09	5.18	4.82	5.1	4.55
13	4.21	4.66	5.66	6.82	5.34	3.32	5.14	5.76	6.02	5.06
15	4.37	5.92	4.59	6.9	5.45	4.33	5.01	6.18	5.2	5.18
18	3.85	6.12	4.4	7.4	5.44	4.07	4.67	5.14	6.12	5.00
22	4.38	4.30	5.87	7.1	5.41	2.27	6.08	7.84	5.4	5.39
25	4.42	5.2	4.25	7.8	5.42	2.34	4.86	5.54	5.8	4.64
30	5.36	4.5	4.34	5.21	4.85	2.97	5.79	4.84	3.5	4.27
35	3.29	5.12	4.58	4.69	4.42	2.13	4.92	4.43	2.6	3.52

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TABLE 24.CREATINE PHOSPHOKINASE CONCENTRATIONS. EXPERIMENT 2, SET 2.

	Horse no and treatment									
	Added fat					Control				
Time	2	6	4	8	Avg	1	3	5	7	Avg
min	Sigma units									
0	13	17	12	15	14	26	19	12	7	16
8	19	17	16	18	17.50	20	22	20	8	17.50
13	12	27	17	13	17.25	22	17	13	12	16.00
15	13	16	16	19	16.00	14	14	11	16	13.75
18	11	13	6	22	13.00	21	14	21	28	21.00
22	10	14	12	19	13.75	18	17	19	22	19.00
25	10	18	11	10	12.25	13	8	14	20	13.75
30	11	14	13	11	12.25	13	12	15	11	12.75
35	8	15	10	3	9.00	9	10	10	17	13.00

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