DIETARY FAT AND EXERCISE CONDITIONING EFFECT ON METABOLIC PARAMETERS IN THE HORSE


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Summary

Four isocaloric diets containing 4, 8, 12 and 16% dietary fat (as soybean oil) were fed to four horses at four intervals according to a Latin square design. After 3 weeks of conditioning at each interval, diet effects were evaluated by trotting all horses at 3.2 m/sec for 6 hours. Pre- and posttrotting responses were measured in muscle and liver glycogen, serum long-chain fatty acids, serum electrolytes, serum enzymes, serum cholesterol, plasma glucose, packed cell volume and hemoglobin. Dietary fat was highly correlated with exercise-induced plasma glucose changes and with cholesterol concentrations. Regardless of the diet, linoleate concentration was about eight times higher than that of the other fatty acids, and it increased slightly as dietary fat levels increased. Stearate concentration also increased with increasing dietary fat but palmitic and oleic acid decreased. Increases in fat intake also resulted in slight increases in liver glycogen at the resting level. Conditioning resulted in a significant decrease in exercise-induced fluctuations of serum enzymes and electrolytes but significantly increased elevations of plasma long-chain fatty acid concentrations. Resting muscle glycogen increased by 37% during the study as a result of conditioning, but there was no effect on liver glycogen at rest or after exercise. Feeding of the four levels of dietary fat in the form of soybean oil had no adverse effects and proved a safe and efficient method of providing concentrated energy to working horses.

(Key Words: Horses, Diet Fat, Serum Metabolites, Muscle Metabolites, Conditioning Effects.)

Introduction

Endurance competition with horses is one of the fastest growing equestrian sports but only limited research has been conducted on this subject. A few studies have measured blood metabolites during endurance rides (Mansmann et al., 1973; Carlson and Mansmann, 1974, Rose et al., 1977), and some have described metabolite changes during a training regimen (Goodman et al., 1973; Haynes, 1974; Snow and McKenzie, 1977). Nearly all studies have involved relatively short training and work intervals and, therefore, have only limited application given that many hours of riding take place in actual competition. Only one study has attempted to relate nutritional considerations to prolonged submaximal exercise in the horse (Slade et al., 1975). The high levels of carbohydrate and protein commonly fed in grain mixes to meet energy requirements of endurance horses can sometimes lead to azoturia and other digestive disturbances. These diets may not provide maximal nutritional and physiological preparation of the horse for long-term aerobic work. The need for more information from controlled experiments about what to feed horses and for the dissemination of this information was demonstrated by a nationwide survey of endurance riders which indicated that competitors were unfamiliar with available information about nutritional adaptations for endurance type work (P. L. Hambleton, unpublished data). Most respondents believed that high protein diets were best suited for this type of activity.

The objective of the experiment described herein was to determine the effects of different dietary ratios of carbohydrate and fat fed...
at the same caloric intake on serum and tissue metabolites in horses used for prolonged submaximal exercise.

Methods

Routine. One gelding and three mares ranging in age from 4 to 11 years were each fed one of four levels of dietary fat in a $4 \times 4$ Latin square design (table 1). Each horse was evaluated before and after prolonged periods of submaximal exercise. The horses were kept in individual $10 \times 35$ m barren paddocks equipped with automatic waterers. The horses were fed twice daily. Hay was fed in a manger and grain in formed rubber feeding buckets to minimize oil losses. The study was conducted during the winter and early spring when the mean ambient daily temperature was approximately 0 C.

Time Periods and Trials. Each experimental period lasted 3 weeks, during which the horses were conditioned for 2 hr/day. For 2 to 3 days/week, the conditioning was done with a mechanical horse walker set for 3.2 m/sec at the trot; on 2 or 3 other days each week, the horses were ridden at a trot for 2 hr in the surrounding foothills. The total number of conditioning days per week was five. At the end of each 3-week period, an exercise trial was conducted which consisted of trotting the four horses at 3.2 m/sec on the mechanical walker for 6 hours. At the end of each hour, the horses were allowed to walk for 5 min and to drink water. The mechanical walker was used to ensure uniform amounts of stress on each horse during each test period.

Diets. Dietary fat levels of 4, 8, 12 and 16% were attained by the addition of soybean oil to a basal diet. Energy intake was balanced between diets by the removal of corn and addition of fat (table 2). The oil was mixed with the grain just before each feeding in the am and pm. The number of calories fed was sufficient to meet the requirements of 2 hr work/day and to compensate for heat loss during cold weather. Caloric sufficiency was evaluated in terms of weight maintenance.

Measurements. Immediately before and after each 6-hr exercise trial, samples were taken from all horses for analysis. Blood was collected by jugular puncture into clot and heparinized vacuum tubes, and biopsies were taken from the quadriceps femoris muscle and from the liver; by needle the liver biopsies were collected through the 13th intercostal space after local anesthetization with lidocaine hydrochloride. The heparinized samples were kept in an ice bath until one aliquot was analyzed for packed cell volume and hemoglobin. Plasma collected from another aliquot was stored at $-4$ C and then analyzed for fatty acids on a gas chromatograph by the method of Metcalfe and Schmitz (1961). Serum was collected from the clot tube and stored at $-4$ C for chemical analysis. Glycogen was determined by the method of Passonneau et al. (1967). Blood samples were analyzed on a Hycel 17 autoanalyzer for glucose, calcium, inorganic phosphorus, sodium, potassium, chloride, cholesterol, packed cell volume, hemoglobin,

<table>
<thead>
<tr>
<th>TABLE 1. SEQUENCE OF HORSE EXPOSURE TO EACH OF FOUR LEVELS OF DIETARY FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary fat (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>16</td>
</tr>
</tbody>
</table>

*Each time period lasted 3 weeks, during which exercise conditioning and dietary adaptation took place.*

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6 Sterling and Sons, Denver, CO, 80222.
7 Varian Aerograph 1700, 611 Hansen Way, Palo Alto, CA, 94300.
8 Hycel, Inc., Houston, TX.
TABLE 2. DIET COMPOSITION

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>4% fat</th>
<th>8% fat</th>
<th>12% fat</th>
<th>16% fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oats</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Grass hay</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Supplement&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.23</td>
<td>.23</td>
<td>.23</td>
<td>.23</td>
</tr>
<tr>
<td>Corn, flaked</td>
<td>1.10</td>
<td>.82</td>
<td>.63</td>
<td>.42</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>.06</td>
<td>.17</td>
<td>.26</td>
<td>.35</td>
</tr>
<tr>
<td>Trace mineral salt&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.019</td>
<td>.019</td>
<td>.019</td>
<td>.019</td>
</tr>
<tr>
<td>Total kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.909</td>
<td>2.739</td>
<td>2.639</td>
<td>2.519</td>
</tr>
</tbody>
</table>

<sup>a</sup>All American Horse Supplement, Ranch Way Feedmill, Fort Collins, CO 80524.

<sup>b</sup>Mornan's Trace Mineral Salt.

<sup>c</sup>Each diet provided 3.7 Mcal digestible energy and 160 g digestible protein per 45.4 kg body weight of horse per day.

lactate dehydrogenase (LDH), alkaline phosphatase (AP), creatinine phosphokinase (CPK), serum glutamic-pyruvic transaminase (GPT) and serum glutamic-oxalacetic transaminase (SGOT).

Statistics. Analysis of variance was determined for a Latin square design (Rickimers and Todd, 1967). The least significant difference test (Steel and Torrie, 1960) was used to identify different means.

Results

Training Effects. Training lowered (P<.05) the resting levels of long-chain fatty acids in the plasma (table 3). All plasma long-chain fatty acids initially decreased with exercise, but with improved physical conditioning, they subsequently increased (figure 1). Glucose followed a similar pattern, being depressed during the first period of exercise and increased progressively after the second, third and fourth periods (figure 2). Training had no effect on liver glycogen concentrations (figure 2). Conditioning reduced (P<.05) exercise-induced increases in SGOT (r=-.98) and CPK (r=-.97) (figure 3). LDH and GPT were not affected (table 3). The resting plasma inorganic phosphorus concentration increased (P<.05) by 70.9% during the 12 weeks, while resting calcium concentrations were unchanged. However, training resulted in a greater exercised-induced decrease in calcium concentration (P<.05, r=-.97, table 3). Exercise-induced increases in the other electrolytes decreased nonsignificantly with training (table 3). Cholesterol levels did not increase with training, either at rest or after exercise (figure 2).

Dietary Effects. Dietary fat was highly correlated (r=.93) with pre- and postexercise cholesterol concentrations, although all concentrations remained within a normal range and the mean values were not significantly different (figure 4). Elevation of plasma glucose following exercise was highly correlated with increasing dietary fat (r=.91) (figure 5). The increase in glucose after exercise was 58% greater for horses fed the 16% fat diet than for those on the 4% fat diet. No dietary effect on either serum enzymes or serum electrolytes could be detected before or after exercise (table 4). Increased dietary fat resulted in increased resting levels of stearate (r=.89) and linoleate (r=.78) but decreased palmitate and oleate concentrations (figure 6). Resting concentrations of muscle glycogen increased nonsignificantly with the lower levels of dietary fat but decreased at the 16% fat level (figure 4). Resting levels of liver glycogen increased by 15% when dietary fat was increased from the 4 to 16% although this increase was not significant (figure 4).

Discussion

The primary reason for feeding high fat diets is to enable the high performance horse to ingest a concentrated energy diet that is highly digestible. Dietary fat is readily digestible by the horse even at high levels. In one study, fat


### TABLE 3. THE INFLUENCE OF CONDITIONING ON METABOLITES OF HORSE DURING EXERCISE

| No. of weeks of conditioning | Exercise | | | | | |
|-----------------------------|----------|----------|----------|----------|----------|----------|----------|
|                             | Before   | After    | Before   | After    | Before   | After    | Before   | After    |
| LDH, U/liter                | 196.0    | 194.0    | 226.0    | 229.0    | 242.0    | 211.0    | 242.0    | 239.0    |
| CPK, U/liter                | 45.3     | 78.3     | 39.3     | 43.8     | 52.0     | 52.5     | 194.0    | 176.0    |
| AP, U/liter                 | 51.0     | 77.0     | 49.7     | 54.7     | 50.8     | 55.0     | 63.8     | 69.5     |
| SGOT, U/liter               | 204.0    | 239.0    | 165.0    | 186.0    | 180.0    | 198.0    | 198.0    | 258.0    |
| GPT, U/liter                | 29.3     | 27.5     | 24.5     | 30.3     | 42.8     | 26.8     | 97.0     | 45.8     |
| Serum Electrolytes          |          |          |          |          |          |          |          |          |
| Na, meq/liter               | 139.0    | 140.3    | 137.1    | 140.2    | 138.5    | 139.0    | 137.9    | 142.5    |
| K, meq/liter                | 5.5      | 5.6      | 3.9      | 3.8      | 4.7      | 4.4      | 7.9f     | 4.3      |
| Cl, meq/liter               | 102.5    | 103.0    | 96.0     | 98.1     | 94.0     | 92.3     | 100.1    | 100.5    |
| Pi, mg/dl                   | 2.7      | 3.5      | 2.7      | 3.0      | 4.0      | 4.2      | 4.6f     | 4.8      |
| Ca, mg/dlab                 | 12.7     | 12.4     | 12.4     | 11.1     | 12.8     | 10.5     | 15.3     | 10.6     |
| Plasma Fatty Acids          |          |          |          |          |          |          |          |          |
| C16, mg/dlbc                | 4.7      | 3.8      | 3.6      | 2.9      | 3.5      | 4.4      | 3.4      | 4.3      |
| C18, mg/dlbc                | 5.7      | 4.7      | 4.3      | 3.9      | 4.8      | 5.2      | 4.4      | 5.0      |
| C18:1, mg/dlb               | 4.4      | 3.1      | 3.3      | 2.4      | 3.1      | 4.0      | 3.0      | 4.1      |
| C18:2, mg/dlbc              | 25.6     | 18.5     | 18.6     | 14.9     | 18.2     | 19.9     | 17.0     | 18.0     |
| PCV%                        | 37.8     | 38.3     | 35.0     | 35.8     | 34.3     | 35.8     | 37.3     | 39.3     |
| Hgb%                        | 12.8     | 13.3     | 12.1     | 12.3     | 11.5     | 12.1     | 12.9     | 13.8     |

*apreexercise means are different (P<.05).*

*bIncreases due to exercise are different (P<.05).*

*cPostexercise means are different (P<.05).*

*dTrotting for 6 hr at 3.2 m/sec.

e2 hr/day, 5 days a week trotting.

*fExcessive elevation due to partial sample hemolysis.

at dietary levels of up to 20% was 90% digested (Bowman et al., 1977). The goal is to defer the onset of fatigue through increased fat utilization. The current practice of feeding high levels of carbohydrates requires knowledgeable management and careful observation of individual animals to reduce digestive disturbances, founder and azoturia.

**Long-chain Fatty Acids.** The work of Robb et al. (1972) showed the major fatty acids in the horse carcass to be (in decreasing order): oleate, palmitate and linoleate. Other fatty acids (stearate, linolenate, palmitoleate and myristate) were present in ranges of 3 to 10% of the total. The present study of horse plasma showed many similarities, with the major difference the high levels of stearate. Soybean oil contains large amounts of linoleate (52%), oleate (27%) and palmitate (12%) (Hathaway, 1977).

It is doubtful that plasma stearate was formed from the soybean oil directly, although it increased with increasing dietary fat (figure 6). The probable source of stearate was micro-
bial hydrogenation of the oleate and linoleate in the cecum. Serum palmitate concentration decreased with increasing amounts of oil in the diet. This may be explained by the formation of endogenous palmitate from grain carbohydrate in the low fat diets through acetyl-CoA fragments (table 2). The slight decrease in oleate in the plasma with increasing amounts of fat may indicate that this fatty acid was formed by hydrogenation in the cecum. Linoleate increased directly in relation to the amount of oil in the diet. It was by far the major fatty acid in the plasma and was six times higher than any of the other fatty acids.

**Plasma Glucose.** Small decreases to slight increases in plasma glucose have been reported to occur in horses during exercise, depending on the duration and intensity of the exercise. Low blood glucose, which usually occurs after 3 or 4 hr of prolonged exercise, has frequently been associated with fatigue in the horse (Carlson et al., 1965; Lindholm and Piehl, 1974; Lindholm et al., 1974; Lindholm and Saltin, 1974). Liver glycogen was also found to be depleted at this time. In the present study, both increases and decreases in liver glycogen

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**Figure 2.** Influence of conditioning on serum and muscle metabolites in horses.

**Figure 3.** The influence of conditioning on the elevation of serum enzymes during exercise. (U/L, units per liter; CPK, creatinine phosphokinase; AP, alkaline phosphatase; SGOT, serum glutamic-oxalacetic transaminase).
with exercise were observed. In the untrained animals, the plasma glucose concentration decreased with exercise, while in the trained animal, it increased. Johnson and Rennie (1973) obtained similar results in a study with humans. The extent of the increase in plasma glucose with exercise depended not only on conditioning but on dietary fat levels, as well ($r=0.91, P<.05$). Increases in plasma glucose after exercise in horses fed dietary fat have been reported (Slade et al., 1975). In the present study, the increase in plasma glucose was 58% greater in exercised horses on the 16% fat diet than in those on 4% fat. The importance of this observation is not clear, but if central nervous system function is impaired by low blood glucose at the concentrations encountered in endurance work, then the increased glucose resulting from the feeding of high fat diets may be of practical importance. Training has been shown to increase the utilization of fat as an energy source (Mager et al., 1964; Goodman et al., 1973; Mole et al., 1971). Goodman et al. (1973) found that unconditioned horses used fat and carbohydrate as energy sources but that conditioned horses exhibited a greater utilization
and a greater ability to oxidize fat. Holloszy (1967) found that the enzymes of lipid metabolism in humans increased twofold with training and that carbohydrate utilization was reduced. From this it would appear that carbohydrate loading, as suggested for humans by Bergström and Hultman (1972), would be contrary to some of the adaptations of the horses' muscle to submaximal exercise. It could be a dangerous practice with horses, as it might induce azoturia and muscle spasms and tetany.

Mole et al. (1971) found that palmitate, oleate and linoleate oxidation capability by rats increased twofold with conditioning. Training increased the animals' fat utilization and decreased their use of glycogen for energy. Increased fat utilization spared glycogen and left blood glucose for central nervous system function. The increased fat utilization suggested by Mole et al. (1971) agrees with the progressive increase in fatty acids during exercise in trained horses in the present study.

The effect of soybean oil in the diet was very likely masked in some of the other measurements. This masking was due to the increase in body fat synthesized from the excess carbohydrate. It has been suggested that the shorter chain fatty acids may be better utilized than the higher molecular weight, long-chain fatty acids because of easier transport (Scott et al., 1976). If this is true, some of the beneficial aspects of feeding soybean oil or other fat would be lost if the shorter chain fatty acids synthesized from carbohydrates were better utilized by the working muscle.

**Glycogen.** The mean liver glycogen content before exercise was 414 mmoles (glucose units)/kg dry tissue weight. This concentration agrees with those reported by Lindholm et al. (1974) when their values are expressed on a dry weight basis. During exercise, there was a 38% reduction in the glycogen concentration. Although horses on the 16% fat diet had the highest postexercise liver glycogen concentrations, the concentrations were not significantly different from those of the other animals. Conditioning did not significantly affect liver glycogen concentrations. For muscle glycogen at the preexercise resting level, a significant conditioning effect could be seen only between the first and last time periods (213 vs 290 mmoles [glucose units]/kg dry tissue). Increased dietary fat resulted in 46% higher muscle glycogen concentrations in pretrotted horses up to the 12% fat diet. Glycogen concentrations in horses fed the 16% fat diet, however, did not differ significantly from the concentrations in those fed 4% fat. The mean muscle glycogen values before exercise were 245 mmoles (glucose units)/kg dry tissue. This
Figure 6. Influence of dietary fat level on serum-free fatty acids in horses.

Figure is slightly lower than those in previous reports on horses (Lindholm et al., 1974). This difference may be due to the short period of training for the horses in this study compared with that for the racing animals about which previous reports were made.

It could be concluded from this study that at the speed of 3.2 m/sec for 6 hr, glycogen depletion in the liver and muscle would not be a factor in fatigue. This conclusion is supported by experiments by Lindholm et al. (1974) in which Standardbred trotters were trotted for 4 hr, and muscle glycogen never declined to levels that should have impaired performance, yet the horses were fatigued and unwilling to continue, suggesting a different cause of exhaustion. It should be kept in mind that in actual endurance competition, fast starts or periods of quick acceleration may deplete glycogen rapidly.

Serum Enzymes. The primary and significant effects on serum enzymes were due to conditioning. Apparently, the animals were not in peak condition during the first trial, as large increases in the enzymes occurred. The last three trials showed much less change in enzyme concentrations, indicating that the animals were in better condition or that there was less stress on their systems (figure 3). This observation is in agreement with work by Anderson (1975) and Kronfeld et al. (1978), who reported diminished increases in CPK, LDH and aldolase (ALD) with training. The decreases observed in the LDH and GPT levels with training probably indicate a clearing from the serum during exercise, with increased cardiac output and increased membrane stability.

Diet had no effect on any of the serum enzyme concentrations of horses, either in the resting state or during exercise. One might have expected a change in the LDH concentration with the change in the amount of carbohydrate in the diet. This was not observed, possibly because of the lack of specificity of the enzyme.

Of the five enzymes measured, two (GPT, SGOT) are sensitive indicators of muscle function and one, CPK, is fairly specific and sensitive. Alkaline phosphatase and LDH
are nonspecific and may be increased during acute cellular damage of many tissues (Gerber, 1969; Curtis, 1974; Coodley, 1975). None of the enzymes has known functions in the serum, although LDH and AP are abundant in red blood cells and are released during hemolysis or periods of membrane instability.

**Serum Electrolytes.** There was a remarkable constancy in the serum concentrations of all electrolytes measured in relation to the diets. This would be expected from homeostasis and because most of the basic minerals were fed to all horses at the same level. Diets had no significant effect on the preexercise or post-exercise levels of any of the electrolytes.

Training appeared to decrease the exercise-induced increases in potassium \((r = .84)\) and phosphorus \((r = .90)\). The depletion of calcium appeared to be greater with training \((r = .97)\). Sodium and chloride levels before and after exercise did not vary with training. The pre-exercise level of inorganic phosphorus increased significantly with training, to almost twice the initial level \((r = .95)\). Such increases have previously been attributed to increased ATP activity in the muscle (Holloszy, 1967).

Sodium, chloride and potassium are the main electrolytes maintaining osmotic balance in body fluids. In studies of endurance horses, large quantities of sodium and chloride have been lost in the form of sweat (Carlson and Mansmann, 1974). In the present study, cool temperatures and level terrain kept sweating to a minimum. This was reflected in the post-exercise concentrations of electrolytes, which remained fairly normal compared with those observed in horses that have perspired considerably (Rose et al., 1977).

The changes in the potassium concentrations can not be ascribed entirely to losses in sweat. In one instance, partial hemolysis was evident (table 3) and that could have accounted for the excessively high serum potassium concentration. The postexercise level of potassium in the first trial was significantly higher than that in the last three trials, probably because of increased muscle cell membrane permeability in the untrained animal, as was inferred from the serum enzyme concentrations. After exercise, potassium decreased significantly, which reflects increased membrane stability with increased physical conditioning.

Exercise reduced the serum concentration of calcium. The calcium depletion was significantly greater in the trained horses at the end of the study. The resting concentration of calcium did not change with training. A severe decrease in serum calcium concentration in the horse results in muscle faniculations and tetany. The horses dietary requirements of calcium have not been found to increase with exercise, perhaps because urinary retention may be increased (Schryver et al., 1975). The most important aspect of calcium in exercise is that of muscle and nerve membrane potential, and this element is necessary for the depolarization of the sarcoplasmic reticulum to initiate muscle contraction. Since sweat may contain appreciable amounts of calcium, further study is needed to determine calcium metabolism during exercise as affected by intensity and duration of exercise and the limits of increased dietary retention with respect to the nutritional requirement.

**Packed Cell Volume and Hemoglobin.** There were no significant conditioning or dietary effects on either packed cell volume or hemoglobin. Although many professional trainers relate packed cell volume to conditioning, this supposition has not been substantiated in scientific studies. The small increases during exercise in the present study indicated good hydration of the horses as a result of minimal sweating and the animals chance to drink every hour.

**Serum Cholesterol.** All horses had normal serum cholesterol concentrations although the concentrations were highly correlated with dietary fat \((r = .93, P < .05)\). These results do not support Goodman et al. (1973), who reported that conditioning increases the resting concentration of cholesterol in the serum. Cholesterol in horses has previously been shown to increase as a result of exercise (Goodman et al., 1973; Rose et al., 1977), as was observed in this study.

**Literature Cited**


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