Effects of feeding meals with various soluble-carbohydrate content on muscle glycogen synthesis after exercise in horses

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Objectives—To determine effects of feeding diets with various soluble-carbohydrate (CHO) content on rates of muscle glycogen synthesis after exercise in horses.

Animals—7 fit horses.

Procedures—In a 3-way crossover study, horses received each of 3 isocaloric diets (a high soluble CHO [HC] diet, a low soluble CHO [LC] diet, or a mixed soluble CHO [MC] diet). For each diet, horses were subjected to glycogen-depleting exercise, followed by feeding of the HC, LC, or MC diet at 8-hour intervals for 72 hours.

Results—Feeding the HC diet resulted in a significantly higher glycemic response for 72 hours and significantly greater muscle glycogen concentration at 48 and 72 hours after exercise, compared with results after feeding the MC and LC diets. Muscle glycogen concentrations similar to baseline concentrations were detected in samples obtained 72 hours after exercise in horses when fed the HC diet. Rate of glycogen synthesis was significantly higher when horses were fed the HC diet, compared with values when horses were fed the MC and LC diets. Glycogen synthase activity was inversely related to glycogen content. Protein content of glucose transporter-4 was the lowest at 72 hours after exercise when horses were fed the HC diet.

Conclusions and Clinical Relevance—Muscle glycogen synthesis was slower after glycogen-depleting exercise in horses, compared with synthesis in humans. Feeding HC meals after strenuous exercise hastened replenishment of muscle glycogen content, compared with results for feeding of LC and MC diets, by increasing availability of blood glucose to skeletal muscles. (Am J Vet Res 2004;65:916–923)

Glycogen is the principal energy source for skeletal muscles during vigorous exercise. In horses and humans, depletion of intramuscular glycogen stores is associated with hastened fatigue during endurance exercise and high-intensity exercise. High-intensity exercise in Thoroughbred or Standardbred racehorses depletes muscle glycogen concentrations by 30% to 40%, and participation of endurance horses in 80- to 160-km races depletes muscle glycogen content by 50% to 75%. Moreover, complete replenishment of muscle glycogen stores after exercise requires 48 to 72 hours. Given the substantial muscle glycogen depletion associated with exercise, the slow rate of replenishment, and the fact that many horses participate in several events in a single day, the interval between exercise bouts appears to be inadequate for complete restoration of the muscle glycogen pool. Therefore, enhanced replenishment of energy substrate stores after exercise may confer substantial advantages for the performance of equine athletes.

Effects of diet composition on availability of muscle glycogen and exercise capacity have received much attention in human exercise physiology and have resulted in substantial improvements in nutrition and performance of human athletes. Variations in glucose responses after ingestion of carbohydrate diets has led to the establishment of the glycemic index, which classifies dietary carbohydrates on the basis of the increase in blood glucose relative to an equivalent amount of carbohydrate ingested in the form of glucose. Ingestion of foods with a high glycemic index by humans after exhaustive exercise increased the rate of muscle glycogen synthesis 2-fold by 6 hours after exercise, compared with results for a meal with a low glycemic index. Furthermore, feeding a meal with a high glycemic index increased muscle glycogen concentration 2-fold by 24 hours after glycogen-depleting exercise, compared with results for a meal with a low glycemic index; however, this effect is not consistent.

Although the effects of dietary manipulation on availability of muscle glycogen have been investigated in human exercise physiology, there is little information regarding dietary factors affecting muscle glycogen synthesis after exercise in horses, and the conclusions from human studies cannot be applied to horses because of the physiologic differences between these species, especially with regard to carbohydrate metabolism. For instance, horses have greater concentrations of muscle glycogen when exposed to similar dietary and training conditions and a greater relative (per kilogram of body weight) capacity for energy production via aerobic and anaerobic metabolism compared with results for humans. Perhaps of most importance, muscle glycogen synthesis appears to be slower in horses compared with other species, although no mechanistic explanation has
yet been provided. Because of the slow rate of muscle glycogen synthesis, composition of diets fed after exercise may be crucial to maximize replenishment of muscle glycogen stores in horses. Whereas IV infusion of glucose (6 g/kg) hastens replenishment of muscle glycogen stores, compared with results after infusion of saline (0.9% NaCl) solution, none of the studies conducted has conclusively documented an effect of diet with various soluble-carbohydrate content on rates of muscle glycogen synthesis in horses after exercise. Therefore, our objectives for the study reported here were to measure the rate of glycogen synthesis, protein content of glucose transporter (GLUT)-4, and muscle glycogen synthase activity in horses and compare the effects of feeding 3 isocaloric diets of various soluble-carbohydrate content after exercise on replenishment of muscle glycogen stores. Our hypotheses were that muscle glycogen synthesis would be slow after strenuous exercise as a result of the limited activity of glycogen synthesis enzymes or decreased protein content of GLUTs and that feeding a diet high in soluble carbohydrates would hasten replenishment of muscle glycogen stores after exercise, compared with results after feeding diets lower in soluble carbohydrates.

Materials and Methods

Animals—Seven Standardbred gelding horses (2 to 10 years old; mean ± SE body weight, 473 ± 9 kg) that had mean body condition scores of 4.6 ± 0.3 were used in the study. Horses were chosen on the basis of aerobic capacity for that horse. Horses ran on an inclined treadmill (slope, 4°) for 5 minutes at a rate of 4 m/s, 15 minutes at a rate calculated to achieve 70% of V_o,max, and 5 minutes at a rate calculated to achieve 90% of V_o,max. Horses were allowed to rest for 30 minutes. Then, they completed 6 sprints (1 min/sprint) at a rate calculated to achieve 100% of V_o,max; horses were walked for 5 minutes between each sprint. Other studies have documented that this exercise protocol depletes muscle glycogen stores by at least 60% of the initial value. Feed was withheld for 12 hours before the onset of strenuous exercise, and horses were fed 8.5 kg of timothy grass and alfalfa hay/d during the 3 days of exercise.

Dietary intervention—Immediately after the last bout of exercise in each experiment, the horses received an isocaloric high soluble carbohydrate diet (grain; diet HC), an isocaloric low soluble carbohydrate diet (hay; diet LC), or an isocaloric mixed soluble carbohydrate diet (grain and hay; diet MC; Appendix 1). The MC diet was considered the control diet. The order in which the diets were fed was randomized. Thus, at the end of the 3 experiments, the 7 horses had each received each of the 3 diets. Diets were fed at 8-hour intervals for 72 hours (days 3 to 5). Digestible energy (DE) for each diet was calculated on the basis of the daily energy requirement for horses performing light work. Samples of the diets were retained, and a commercial laboratory conducted an analysis on the samples (Appendix 2). On the basis of this analysis, the amount of each diet fed to each horse was calculated (HC diet, 6.5 ± 0.2 kg/horse/d; MC, 7.8 ± 0.2 kg/horse/d; LC diet, 9.7 ± 0.3 kg/horse/d). The amount of feed and water consumed was recorded for each horse during the 72 hours of dietary interventions. During the experimental period, horses were housed separately in box stalls and walked twice daily for 10 minutes.

Collection of muscle biopsy specimens—Muscle specimens were collected by use of needle biopsy from the middle gluteal muscle. Specimens were collected from 8 sites in the left or right middle gluteal muscles. Sites for muscle biopsy were marked by use of an indelible marker and a grid pattern. Biopsies were collected at a uniform depth. Muscle samples were collected before exercise (day 1) and 0, 3, 6, 12, 24, 48, and 72 hours after the bout of exercise (day 3). Muscle samples were flash-frozen in liquid nitrogen and stored at −80°C until subsequent analysis of muscle glycogen concentration, glycogen synthase activity, and total protein content of GLUT-4. Muscle glycogen concentrations did not differ significantly among samples obtained from the various sites.

Collection of blood samples—Blood samples were collected via a 14-gauge, 5.25-inch catheter inserted into a jugular vein. Skin overlying the jugular was desensitized by injection of 2% mepivacaine. For each experiment, muscle samples were collected from 8 sites in the left or right middle gluteal muscles. Sites for muscle biopsy were marked by use of an indelible marker and a grid pattern. Biopsies were collected at a uniform depth.

Experimental design—Effects of dietary carbohydrate administration on muscle glycogen synthesis were examined by use of a partially balanced, 3-way crossover design. All 7 fit horses performed each of the 3 experiments; experiments were separated by 10-day intervals.

Glycogen-depleting exercise—All horses performed 3 days of strenuous exercise intended to deplete muscle glycogen stores by at least 60% of the initial values (days 1 to 3).

The exercise protocol was customized for each horse on the basis of aerobic capacity for that horse. Horses ran on an inclined treadmill (slope, 4°) for 3 minutes at a rate of 4 m/s, 15 minutes at a rate calculated to achieve 70% of V_o,max, and 5 minutes at a rate calculated to achieve 90% of V_o,max. Horses were allowed to rest for 30 minutes. Then, they completed 6 sprints (1 min/sprint) at a rate calculated to achieve 100% of V_o,max; horses were walked for 5 minutes between each sprint. Other studies have documented that this exercise protocol depletes muscle glycogen stores by at least 60% of the initial value. Feed was withheld for 12 hours before the onset of strenuous exercise, and horses were fed 8.5 kg of timothy grass and alfalfa hay/d during the 3 days of exercise.
Body weight—All horses were weighed before exercising on days 1 and 3; horses were weighed each day during the treatment periods. Body condition scores were recorded before and after each experiment by use of a National Research Council scoring system.17

Muscle glycogen content and glycogen synthase activity—Following acid hydrolysis, muscle glycogen concentrations were determined in duplicate by use of a fluorometer.18 For determination of glycogen synthase activity, samples from gluteal muscle were homogenized (1:50) in a solution that contained 30% glycerol, 20mM phosphate buffer (pH, 7.4), 5mM 2-mercaptoethanol, 0.5mM EDTA, and 0.02% bovine serum albumin. Glycogen synthase activity was then measured fluorometrically without (active form) or with (total activity) addition of 10mM glucose-6-phosphate.19 Rate of glycogen synthase activity was reported, and the activity ratio (active:total) was calculated.

Total protein content of GLUT-4—Total crude extract of muscle membranes was obtained as described elsewhere,20,21 and analyzed for GLUT-4 content by use of electrophoresis and subsequent immunoblotting with polyclonal antibodies directed against rabbit GLUT-4 and, subsequently, anti-rabbit horseradish peroxidase antibody.21,22 Crude membrane preparations of equine cardiac tissues were used as a positive-control standard, and density was expressed as the percentage. This technique has been validated for use in samples obtained from horses.23

Hematologic and biochemical analysis—Plasma glucose and lactate concentrations were measured spectrophotometrically by use of a microplate reader and commercially available kits.21 Serum insulin concentration was measured by use of a radioimmunoassay with a commercial kit validated for use in samples obtained from horses.21 All samples were analyzed in duplicate, except samples used for determination of glucose concentrations, which were measured in triplicate. Hematocrit and plasma total protein concentration were measured by use of the microhematocrit technique and a refractometer, respectively.

Statistical analysis—Statistical analyses were performed by use of a 1- or 2-way repeated-measures ANOVA (repeated measures on time and treatments), as appropriate for the dependent variables. The null hypothesis was rejected at \( P < 0.05 \). Significant differences between means were identified by use of the Student-Newman-Keul test. Statistical analyses were performed by use of a statistical software package. All results were expressed as mean ± SE.

Results

\( \dot{V}_{O_2} \text{max} \) and the ratio of speed to \( \dot{V}_{O_2} \)—The \( \dot{V}_{O_2} \text{max} \) of the horses before the 3-day exercise period in experiment 1 was 109 ± 6 mL of \( \dot{O}_2/\min/kg \) at a treadmill speed of 10.7 ± 0.3 m/s. The ratio of speed to \( \dot{V}_{O_2} \) (0.990 ± 0.003) was significantly \( (P < 0.001) \) correlated. Slope of the regression line was 8.5 ± 0.4 mL of \( \dot{O}_2/\min/kg \), and the ordinate intercept was 3.5 ± 1.4 mL of \( \dot{O}_2/\min/kg \).

Glucose and insulin concentrations—Feeding varying amounts of soluble carbohydrate affected plasma glucose and insulin concentrations (Figures 1 and 2). Before and after exercise, glucose and insulin concentrations were similar for the 3 treatment diets. Feeding the HC diet after exercise resulted in significantly \( (P = 0.005) \) greater plasma glucose concentrations (up to 4 hours after feeding), compared with concentrations after feeding the LC or MC diets; plasma glucose concentrations were similar for the horses when fed the MC (4.7 ± 0.1mM) and LC (4.8 ± 0.1mM) diets. Furthermore, glucose concentrations greater than those measured before exercise were detected from 9 to 72 hours after exercise only in horses when fed the HC diet. The response of serum immunoreactive insulin concentrations to the treatment diets was similar to the response of glucose concentrations to the diets. Feeding horses the HC diet induced a hyperinsulinemic response in which insulin concentrations significantly \( (P < 0.001) \) increased by 70% from 85.7 ± 11.1pM before feeding to 144.1 ± 16.8pM 2 hours after feeding), although insulin concentration when horses were fed the HC diet did not differ significantly from concentrations after the second meal when horses were fed the MC or LC diets. Insulin concentration remained significantly \( (P = 0.005) \) higher at 48 and 72 hours after exercise in horses when fed the HC diet.
Muscle glycogen concentrations—Strenuous exercise resulted in a significant ($P < 0.001$) depletion of muscle glycogen stores for all treatment diets (from $129.0 \pm 3.6$ mmol/kg [wet-weight basis] before exercise to $28.0 \pm 3.6$ mmol/kg after exercise; Figure 3). Muscle glycogen concentrations were significantly ($P < 0.001$) decreased from the initial values (64.4%, 54.2%, and 52.9% of initial values when horses were fed the HC, LC, and MC diets, respectively) 24 hours after exercise. Feeding diets with differing glycemic indices affected muscle glycogen synthesis. Horses fed HC meals had greater muscle glycogen concentration 48 and 72 hours after exercise, compared with concentrations when the horses were fed the MC or LC diets. At 72 hours after exercise, muscle glycogen concentration for horses when fed the HC diet ($133.6 \pm 3.6$ mmol/kg) was significantly ($P < 0.001$) higher than the muscle glycogen concentration when horses were fed the MC ($106.8 \pm 3.6$ mmol/kg) or LC ($102.6 \pm 3.6$ mmol/kg) diets. Overall, the rate of glycogen synthesis during the 72-hour study period was significantly ($P < 0.001$) higher for horses when fed the HC diet ($1.51 \pm 0.15$ mmol/kg/h), compared to the rate when horses were fed the MC ($1.12 \pm 0.11$ mmol/kg/h) or LC ($0.97 \pm 0.10$ mmol/kg/h) diets.

The rate of muscle glycogen synthesis decreased progressively after exercise. It was significantly higher at 3 hours after exercise ($3.33 \pm 0.29$ mmol/kg [wet-weight basis]/h), compared with the rate 12 hours after strenuous exercise ($2.10 \pm 0.29$ mmol/kg [wet-weight basis]/h) for all treatment diets (Figure 4). Rate of glycogen synthesis significantly ($P < 0.001$) decreased further (by 50% and 73%) at 24 and 72 hours after exercise.

Glycogen synthase activity—Glycogen synthase I activity increased significantly ($P < 0.001$) beginning...
immediately after exercise and feeding, with a peak of activity between 3 and 12 hours after exercise (Figure 5). Glycogen synthase I activity decreased in parallel with the rate of glycogen synthesis. Glycogen synthase I activity 48 hours after exercise was similar to baseline activity. Glycogen synthase I activity was higher 3 hours after feeding of the HC diet, compared with activity after feeding of the LC or MC diets. We did not detect significant differences among groups at 3 hours after feeding when results of glycogen synthase activity were expressed as a ratio. There was a strong negative correlation ($R^2$, 0.71; $P < 0.05$) between glycogen content and glycogen synthase activity ratio (Figure 6).

Total GLUT-4 protein content—We detected a significant ($P = 0.001$) effect of time on GLUT-4 protein content. There was not a significant change in GLUT-4 protein content after feeding for up to 48 hours after exercise. In contrast, GLUT-4 protein content was significantly reduced 72 hours after exercise, compared with values up to 48 hours after exercise, in horses when fed the HC diet (Figure 7).

Water consumption, body weight, and body condition scores—The 7 horses consumed all the meals offered. There were no adverse effects of diet observed in the horses during this study. Water consumption differed significantly ($P < 0.001$) among treatments during the 72-hour study period (43.4 ± 3.2, 67.7 ± 7.0, and 88.5 ± 7.5 L for horses when fed the HC, MC, and LC diets, respectively). Body weight was significantly ($P < 0.001$) lower after exercise for all diets (Figure 8). When horses were fed the MC diet, body weight was significantly higher at 72 hours after exercise (460 ± 11.1 kg), compared with body weight at the end of exercise (451.6 ± 8.8 kg). Body weight was significantly lower at 48 and 72 hours after exercise when horses were fed the HC diet, compared with body weight when horses were fed the LC or MC diets. Body condition scores were not significantly different among the experiments (4.6 ± 0.3 before experiment 1, 4.5 ± 0.2 before experiment 2, 4.9 ± 0.3 before experiment 3, and 4.8 ± 0.3 after experiment 3).

Other variables—Plasma concentrations of lactate and total protein and the hematocrit were significantly ($P < 0.001$) higher after the end of exercise, compared with values before exercise (results not shown). We did not detect a significant effect of treatment diet on plasma lactate or total protein concentrations or hematocrit (results not shown).

**Discussion**

We documented that, for the conditions of the study reported here, at least 72 hours was required after strenuous exercise for restoration of muscle glycogen stores in horses fed a diet rich in soluble carbohydrates. Furthermore, feeding a high glycemic index diet (ie, HC diet) hastened muscle glycogen synthesis by increasing blood glucose availability to the skeletal muscle and insulin release, compared with results for feeding of the MC and LC diets.

Our findings on the rate of glycogen synthesis are in accordance with those of other studies in which investigators documented that replenishment of muscle glycogen in horses fed a conventional diet requires...
up to 72 hours after single or repeated bouts of exercise on a racetrack. Although restoration of muscle glycogen stores in human athletes is complete within 24 hours, our muscle glycogen concentration at 24 hours after exercise for horses in our study was 52% to 64% of the before-exercise values. These findings are similar to those of other studies in horses in which negligible or partial repletion of muscle glycogen stores was evident by 24 hours after exercise. The slower repletion of muscle glycogen stores observed in horses, compared with the rate of repletion in humans, is related to the extent of substrate depletion as well as a difference in the rate of glycogen synthesis. For instance, the rate of glycogen synthesis in humans fed after exercise is 6 to 7 mmol/kg (wet-weight basis)/h for the first 3 hours, which is approximately twice the rate of 3.2 mmol/kg (wet-weight basis)/h during the first 3 hours after consumption of the HC diet for horses in the study reported here.

Several factors appear to control muscle glycogen synthesis. The rate-limiting enzyme for glycogen synthesis in skeletal muscles of humans and rats is glycogen synthase, which is regulated by concentrations of muscle glycogen, glucose-6-phosphate, and insulin. Similar to other species, we documented an inverse linear relationship between glucose content and glycogen synthase activity; and the rate of glycogen synthesis decreased in parallel with progressive replenishment of muscle glycogen stores. Furthermore, glycogen synthase activity increased immediately after exercise when glycogen stores were low, suggesting that glycogen availability plays a regulatory role for muscle glycogen synthase by influencing glycogen synthase activity. However, whereas a 5- to 10-fold increase of the glycogen synthase activity ratio has been reported in humans after strenuous exercise, the glycogen synthase activity ratio was only increased 2-fold after exercise in horses in the study reported here. This decrease in glycogen synthase activity observed in horses after exercise could partially explain the slower rate of muscle glycogen synthesis, compared with values reported in humans and rodents. Conversely, continuous replenishment of glycogen stores in the face of low glycogen synthase activity > 48 hours after exercise suggested that other mechanisms, such as GLUT-4 activity or content, influence rate of glycogen synthesis. Surprisingly, GLUT-4 protein content was not significantly affected by exercise and feeding. This finding is in agreement with other studies in which investigators reported no change in GLUT-4 content immediately after exercise. Therefore, the slow rate of replenishment of muscle glycogen stores observed in horses after exercise and feeding may also be related to lower GLUT-4 protein content, compared with GLUT-4 protein content reported in humans and rodents. Finally, muscle glycogen supercompensation, which is defined as a doubling of the concentration of muscle glycogen after glycogen-depleting exercise and carbohydrate feeding, was not observed in the study reported here or in other studies of horses, including after feeding a high carbohydrate diet or after IV infusion of glucose solution (6 g/kg). Because resting muscle glycogen concentration in horses is higher than in humans, we could speculate that high glycogen concentrations in horses exert a negative-feedback signal to stop GLUT-4 recruitment at the plasma membrane, preventing additional muscle glycogen supercompensation.

In agreement with other studies, feeding after exercise induced peaks in postprandial glucose and insulin concentrations within 2 hours after feeding, and blood glucose and insulin concentrations returned to prefeeding values within 5 hours. Furthermore, glucose concentrations after the first meal were not significantly different than the resting values for the 3 treatments, suggesting that exercise attenuates the glycemic response. This decrease in glycemic response in the first hours after exercise is recognized in humans and horses and is attributable to an increase in transport of glucose into skeletal muscles.

The study reported here also revealed that feeding isocaloric diets with varying soluble carbohydrate content after exercise caused differences in glycemic responses. As we hypothesized, feeding a grain-based diet (ie, HC diet) that contained the highest percentage of starch resulted in higher postprandial peaks of glucose and insulin concentrations within 2 hours after feeding, compared with results for the LC and MC diets. Feeding roughage (ie, LC diet) resulted in low to moderate glycemic responses because the bulk of the glucose arises from gluconeogenesis of volatile fatty acids secondary to fermentation in the large colon. Surprisingly, feeding the MC diet resulted in a glycemic response similar to that for the LC diet. It has been documented that feeding hay and grain in the same meal significantly reduces glycemic responses, compared with results for feeding of a grain meal. It was speculated that when feeding a mixed diet, the greater volume of fluid associated with hay consumption (eg, water intake, saliva, and digestive juices) increases the rate of passage of grain through the small intestine and therefore reduces starch digestibility in the small intestine and the postprandial glucose response. In agreement with this theory, water consumption when horses were fed the LC diet was 104% and 31% higher, respectively, compared with consumption reported for horses when fed the HC and MC diets. Furthermore, body weight was significantly higher at 48 or 72 hours after exercise in horses when fed the MC or LC diets, respectively, which probably was attributable to the greater volume of fluid associated with greater hay intake, compared with the volume of fluid associated with the consumption of the HC diet. Therefore, analysis of our results documented that feeding hay (LC diet) and hay along with grain (MC diet) increased water consumption and body weight and reduced glycemic responses, compared with results for the same horses when fed a grain-based diet (HC diet) after exercise.

We documented that feeding an HC diet after exercise hastened muscle glycogen synthesis, compared with results after feeding an isocaloric LC or MC diet. This effect of HC diet on muscle glycogen concentration is associated with increased glycemic responses, suggesting that the increased rate of synthesis of glycogen is attributable to increased glucose availability and uptake by muscles; however, glucose uptake was not
directly measured in this study. Analysis of our results also suggested that increased concentrations of insulin converted glycogen synthase from its inactive form to its active form, which then hastened muscle glycogen synthesis. We speculated that this mechanism was enhanced in horses fed a high glycemic index meal, compared with results when horses were fed a moderate glycemic index meal. However, feeding the HC diet increased glycogen synthase activity only at 3 hours after exercise, compared with activity at 3 hours after exercise for the MC and LC diets.

The effect of feeding the HC diet after exercise on enhancement of muscle glycogen synthesis is in contrast to the effects for carbohydrate provided orally as glucose (3 g/kg or 38 ± 6 g/horse with propionic acid or leucine).21,22 which did not enhance glycogen replenishment after exercise in equine skeletal muscles. In agreement with our findings, a significant increase in muscle glycogen concentration was observed in 6 horses fed a HC diet during a 3-day rest period following glycogen-depleting exercise, compared with values before exercise when horses were in training and fed conventional diets.35 Furthermore, investigators in another study36 documented that muscle glycogen content was higher in horses fed HC and MC diets, compared with results for horses fed an LC diet, only at 28 hours after exercise; however, diets in that study were not isocaloric. Our study revealed that feeding a high glycemic index diet (ie, HC diet) after exercise resulted in greater muscle glycogen concentrations at 48 and 72 hours after exercise and faster rate of glycogen synthesis, compared with results after feeding isocaloric MC or LC diets.

Direct practical implications and applications can be extrapolated from this study. We suggest that horses performing endurance events or horses undertaking several events on successive days could benefit from high soluble carbohydrate diets after exercise to hasten muscle glycogen synthesis. However, because horses fed large amounts of soluble carbohydrate may be predisposed to gastrointestinal disorders,37 optimal nutritional strategies should be developed and careful feeding management ensured.

Appendix 1
Composition of 3 experimental diets fed to horses after a 3-day period of strenuous exercise

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
</tr>
<tr>
<td>Mixed hay</td>
<td>21</td>
</tr>
<tr>
<td>Cracked corn</td>
<td>37</td>
</tr>
<tr>
<td>Oats</td>
<td>20</td>
</tr>
<tr>
<td>Cracked barley</td>
<td>20</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Dry-matter basis.
HC = High soluble carbohydrate diet. MC = Mixed soluble carbohydrate diet. LC = Low soluble carbohydrate diet. – = Not included.

Appendix 2
Estimated analysis of the 3 experimental diets fed to horses after a 3-day period of strenuous exercise

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated starch</td>
<td>50.9</td>
</tr>
<tr>
<td>NDF</td>
<td>22.2</td>
</tr>
<tr>
<td>Protein</td>
<td>8.3</td>
</tr>
<tr>
<td>DE (Mcal/kg/horse)</td>
<td>0.041</td>
</tr>
</tbody>
</table>

*Dry-matter basis.
NDF = Neutral-detergent fiber. DE = Digestible energy. See Appendix 1 for remainder of key.

References

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methods of glycogen measurement in tissues. 


