Effects of low-dose oligofructose treatment administered via nasogastric intubation on induction of laminitis and associated alterations in glucose and insulin dynamics in horses

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Objective—To ascertain whether laminitis can be induced via administration of oligofructose (OF) at doses of 5.0 and 7.5 g/kg in horses and to assess glucose and insulin dynamics before and after treatment.

Animals—19 adult horses.

Procedures—Horses were fed OF (1.0 g/kg) mixed with oats for 6 days. Oligofructose at doses of 5.0 and 7.5 g/kg was then mixed with 4 L of water and administered (0 hours) to 8 (group A) and 4 (group B) horses, respectively, via nasogastric intubation; 8 horses received water alone. One horse in group A that did not develop laminitis was subsequently treated again and included in group B. Before and at intervals after treatment, resting plasma glucose and serum insulin concentrations were measured and frequently sampled IV glucose tolerance tests were performed. Area under the glucose curve (AUCg) and area under the insulin curve (AUCi) were calculated, and minimal model analyses were performed.

Results—3 of 8 horses in group A and all 4 horses in group B developed laminitis. Significant treatment-time effects were detected for resting plasma glucose concentrations and AUCg. Among horses in group A, mean AUCg values at 24 and 48 hours were 34% and 32% higher, respectively, than the mean value at –24 hours. Treatment groups did not differ significantly with respect to resting serum insulin concentration, AUCi, or minimal model analysis results.

Conclusions and Clinical Relevance—In horses, laminitis can be induced and glucose dynamics altered via nasogastric administration of 5.0 g of OF/kg. An alteration in insulin dynamics was not detected following treatment with OF. (Am J Vet Res 2009;70:624–632)

Laminitis is defined as failure of the attachments between the inner hoof wall and the distal phalanx of the foot at the lamellar dermal-epidermal junction. It is a painful and debilitating disease of horses, with major economic and emotional implications to horse owners. In the last National Animal Health Monitoring System survey that was performed in 1998, 13% of horse owners reported that one or more of their horses had developed problems with laminitis within a 1-year period, and 46% of these cases were associated with grazing on pasture.²

Laminitis can be experimentally induced in horses via administration of starch, OF, or soluble extract of black walnut.³–⁵ Starches and fructans are the most abundant forms of nonstructural carbohydrate in pasture grass.⁶ Fructans contain single glucose molecules that are linked to several fructose molecules, whereas starches consist entirely of linked glucose molecules.³ Oligofructose is present in pasture grasses, but the product that is used to experimentally induce laminitis is extracted from the root of the chicory plant (Cichorium intybus) and consists of short-chain, insulin-like fructose polymers.³ Temperate grasses store sugars produced by photosynthesis as fructans, and concentrations increase

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when adverse conditions such as cold or drought slow plant growth and reduce energy consumption. There is also diurnal variation in pasture grass fructan content, with maximum concentrations detected in the late afternoon and evening. Administration of OF induces laminitis in horses by altering the microbial flora of the gastrointestinal tract and is used as a model for pasture-associated laminitis. This experimental model was developed by van Eps and Pollitt, who reported that horses first developed diarrhea and then laminitis after OF was administered at doses of 7.5, 10.0, and 12.5 g/kg. However, the minimum dose of OF required to induce laminitis was not determined, and all of the horses included in that study were euthanized 48 hours after induction of laminitis. The degree of lameness that develops in horses after 48 hours has not been determined for this laminitis induction method, and it has not been established whether horses can recover from the damage caused by OF-induced laminitis.

Insulin resistance is an important risk factor for pasture-associated laminitis; IR is exacerbated by grazing on pasture grass that is rich in nonstructural carbohydrates. However, when laminitis develops, it is difficult to determine whether increased carbohydrate intake exacerbated IR and that this resulted in laminitis or whether gastrointestinal events triggered the disease. The first theory is supported by results of a recent study in which laminitis was induced in healthy ponies via IV administration of insulin. Hyperinsulinemia develops as a result of IR in horses, and it has been shown that blood insulin concentrations increase in grazing ponies as the amount of nonstructural carbohydrates in pasture grass increases. This suggests that laminitis develops in horses that are kept on pasture when IR is exacerbated by dietary changes.

Alternatively, horses that are kept on pasture may develop laminitis as a result of carbohydrate overload, which triggers disease development through the release of factors derived from the large intestine. A systemic inflammatory response is detected subsequent to experimental carbohydrate overload in horses, and this can be attributed to the movement of factors such as endotoxins, exotoxins, and vasoactive amines into the circulation. The same events may be occurring to a lesser extent in ponies and horses that are grazing on pasture. Large quantities of nutrient-rich grass are consumed by horses grazing on pastures in which grasses are growing rapidly as a result of heavy rains, warm weather, or the application of fertilizers. It is conceivable that minor intestinal tract problems develop without detection and that these events trigger laminitis in susceptible animals. These intestinal tract events could also alter glucose and insulin dynamics and exacerbate IR in chronically insulin-resistant equids. The purpose of the study reported here was to ascertain whether laminitis can be induced in horses via administration of OF at doses of 5.0 and 7.5 g/kg and to assess glucose and insulin dynamics before and after treatment. We hypothesized that administration of the lower dose of 5.0 g OF/kg would induce laminitis and alter glucose and insulin dynamics in horses. We anticipated that an experimental protocol could be developed to induce laminitis in horses to an extent from which they would subsequently recover and return to soundness.

Materials and Methods

Animals—Nineteen adult horses (13 mares and 6 geldings) that ranged in age from 3 to 25 years (median age, 10 years) were included in the study; 1 mare was used twice. Breeds of horse included Thoroughbred (n = 5), Appaloosa (3), American Quarter Horse (2), Tennessee Walking Horse (1), and Quarter Horse–Tennessee Walking Horse crossbreds (8). Horses were weighed at the time of admission, and weights ranged from 407 to 582 kg (median weight, 491 kg). None of the horses had acute or chronic laminitis, according to the histories and lameness evaluations. Of the 19 horses, 13 (7 mares and 6 geldings) had been donated for terminal research studies and 6 were from the University of Tennessee teaching and research herd. Only horses donated for euthanasia were included in the OF treatment groups. Reasons for donation included cervical vertebral stenosis (n = 1), narcolepsy (1), chronic uveitis (1), fracture of the distal sesamoid bone (1), arthritis (2), behavioral problems (3), and poor racing or breeding performance (4). The study protocol was approved by the University of Tennessee Instructional Animal Care and Use Committee.

Experimental design—Donated horses were randomly allocated to receive 5.0 g of OF/kg (group A; n = 8) or 7.5 g of OF/kg (group B; 4), and 2 donated horses were included in the control group (group C, 8). Six mares from the university teaching and research herd were included in group C. One donated mare was initially included in group A, but did not develop clinical signs of laminitis after receiving the low laminitis induction dose of OF; after an interval of 2 weeks, this horse was administered the high laminitis induction dose of OF and included in group B.

On admission to the hospital, each horse underwent a physical examination; it was then allowed to acclimate to its environment for at least 60 hours. The horses were housed separately in stalls (3.7 × 3.7 m) within the teaching hospital. Each horse was fed an amount of grass hay that was equivalent to 1.5% of body weight, divided into 4 meals/d. Water was provided ad libitum intake.

Horses were evaluated in groups of 2, and all procedures were performed according to the same schedule. Beginning 6 days before administration of the laminitis induction dose of OF or the control treatment, a diet that consisted of crimped whole-grain oats was fed to each horse in amounts equivalent to 0.5% of body weight (2.5 kg for a 500-kg horse) divided into 2 meals/d. Oligofructose was dissolved in 500 mL of water and mixed with the morning meal of oats; in this manner, each horse received a dose of 1.0 g of OF/kg once daily. During this 6-day period, grass hay was fed as before. An IV catheter was placed in a jugular vein of each horse 2 days prior to induction of laminitis. An FSIGTT was performed the next day (24 hours prior to administration of the laminitis induction dose of OF or control treatment) before horses were fed their morning meal of oats combined with OF. Between 8 AM and...
At 10 AM the next morning, horses were sedated with detomidine hydrochloride (0.005 to 0.01 mg/kg, IV) and administered OF mixed in 4 L of warm water to provide a dose of 5.0 g of OF/kg (group A; n = 8 horses) or 7.5 g of OF/kg (group B; 4 [including 1 horse from group A]) or 4 L of water alone (group C; 8) via nasogastric intubation. Administration of the laminitis induction dose of OF or control treatment was designated as 0 hours. Frequently sampled IV glucose tolerance tests were performed in all horses at 6, 24, 48, and 72 hours.

Assessment of general health—Appearance, attitude, appetite, water intake, rectal temperature, heart and respiratory rates, mucous membrane color, capillary refill time, gastrointestinal tract sounds, and consistency of feces were recorded for each horse in groups A and B at 0 hours (before administration of the laminitis induction dose of OF), every 2 hours until 24 hours, and then every 4 hours until 72 hours after induction of laminitis. Group C horses were not expected to develop laminitis; thus, observations were made at 0 hours and then every 6 hours for the first 72 hours after the horses received the control treatment. For purposes of the study, fever was defined as a rectal temperature ≥ 38.6°C, tachycardia was defined as heart rate > 50 beats/min, and tachypnea was defined as > 30 breaths/min. Laminitis was assessed every 4 hours by use of lameness evaluations and Obel and hoof tester scores (assigned according to established scoring systems). The onset of clinical laminitis was defined as detection of Obel grade 1 lameness. After 72 hours, physical examinations of horses in groups A and B were performed every 12 hours for a maximum interval of 14 days.

Treatment of laminitis—Nonsteroidal anti-inflammatory drug treatment was initiated as soon as clinical signs of laminitis were detected. Horses were administered flunixin meglumine (1.1 mg/kg, IV; q 8 to 12 h), phenylbutazone (2.2 to 4.4 mg/kg, IV or PO; q 12 h), or both.

FSIGTT procedures—Each horse was weighed, and a 14-gauge polypropylene catheter was inserted into the left jugular vein 2 days before treatment at 0 hours. The horse was free to move around the stall and was provided with hay and water. To maintain the patency of the catheter, 5 mL of heparinized saline (0.9% NaCl) solution (10 U of sodium heparin/mL) was administered via the catheter every 4 hours and after every infusion and blood sample collection. An injection cap and infusion set were attached to the catheter during the FSIGTT to facilitate blood sample collection. The insulin-modified FSIGTT first described for use in horses by Hoffman et al was used. Prior to infusion of 50% (wt/vol) dextrose solution (300 mg of glucose/kg) in each horse (duration of infusion, ≤ 2 minutes), blood samples were collected at –10, –5, and 0 minutes (ie, immediately before infusion). Following completion of the dextrose infusion, blood samples were collected via the catheter at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 minutes; insulin (30 mL/kg) was infused at 20 minutes. Blood samples were subsequently collected at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes after dextrose infusion. At each time point, 3 mL of blood was withdrawn from the infusion line and discarded. A 10-mL blood sample was then collected, followed by infusion of 5 mL of heparinized saline solution. Half the volume of blood was transferred to a tube containing sodium heparin, which was immediately cooled on ice and then refrigerated. The remaining blood was transferred to a tube containing no anticoagulant. These samples were allowed to clot at 22°C for 1 hour before serum was harvested via low-speed (1,000 × g) centrifugation. Plasma and serum samples were stored at –20°C until further analyzed.

Blood sample collections—In addition to samples collected during an FSIGTT, blood (10-mL volume) was also collected from the IV catheter as described prior to administration of the laminitis induction dose of OF or control treatment (0 hours), every 2 hours from 0 to 24 hours, and then every 4 to 6 hours from 24 to 72 hours. Blood samples were handled as described.

Assessment of plasma glucose and serum insulin concentrations—Plasma glucose concentrations were measured by use of a colorimetric assay and an automated discrete analyzer. Serum insulin concentrations were determined by use of a radioimmunoassay that has been validated for equine insulin. Each sample was assayed in duplicate, and intra-assay coefficients of variation < 5% or < 10% were required for acceptance of glucose and insulin assay results, respectively. Frequently sampled IV glucose tolerance tests were successfully performed in all horses, but financial constraints limited the number of blood samples analyzed for horses in groups A and C. Measurements of resting glucose and insulin concentrations were limited to blood samples collected at 0 (before administration of the laminitis induction dose of OF or control treatment), 12, 14, 16, 18, 20, 22, 24 (before FSIGTT), 28, 32, 36, 48 (before FSIGTT), and 72 hours.

Interpretation of FSIGTT data—Area under the glucose curve and AUCi values were calculated by use of the trapezoidal method and computer software. For the purposes of these calculations, a mean baseline glucose or insulin concentration was calculated from the measurements made at −10, −5, and 0 minutes during each test. Values for SI, Sg, and AIRg were calculated for each FSIGTT in accordance with the minimal model by use of commercially available software and previously described methods.

Statistical analysis—Mixed-model ANOVA for repeated measures was performed by use of statistical software to examine treatment, time, and treatment-time effects for physical examination variables, resting plasma glucose and serum insulin concentrations, and measures of glucose and insulin dynamics (AUCg and AUCi values, Sg, SI, and AIRg). Groups were also divided according to eventual laminitis status, and this was included in the model as a fixed effect. Significance was accepted at a value of P < 0.05.

Results

With the exception of 1 horse in group A, all horses that received a laminitis induction dose of OF...
developed signs of depression for 28 to 34 hours after OF administration. Inappetence of approximately 48 and 72 hours’ duration was also observed in horses in groups A and B, respectively. Diarrhea was first observed at 14 to 24 hours after administration of the laminitis induction dose of OF in group A and at 8 to 16 hours after administration of the laminitis induction dose of OF in group B. The duration of diarrhea was 6 to 24 hours and 24 to 50 hours in groups A and B, respectively. Fever was detected at 6 to 24 hours after administration of the laminitis induction dose of OF in 7 of 8 horses in group A and in all 4 horses in group B (Figure 1). The duration of fever ranged from 2 to 12 hours and 6 to 16 hours in groups A and B, respectively. A significant ($P = 0.006$) treatment-time effect was detected for rectal temperature; mean values were significantly higher for groups A and B during the 18- to 30-hour period, compared with the value in group C.

Tachycardia was detected in 7 of 8 horses in group A and 2 of 4 horses in group B, and a significant ($P = 0.024$) treatment-time effect was detected for heart rate (Figure 1). Tachypnea was detected in 5 of 8 horses in group A and in 3 of 4 horses in group B, but mean respiratory rate did not differ significantly between groups. None of the horses in the study developed signs of colic following OF administration. No physical examination abnormalities were detected in group B horses.

None of the horses developed clinical signs of laminitis during the 6-day period prior to when 1.0 g of OF/kg was administered once daily in the feed. Three of 8 horses in group A and 4 of 4 horses in group B developed clinical laminitis after administration of the laminitis induction dose of OF, whereas none of the horses in group C developed clinical signs of laminitis. Horses in group A developed laminitis that was associated with Obel grade 1 (n = 1) or grade 2 (2) lameness, and horses in group B developed laminitis that was associated with Obel grade 1 (2), grade 2 (1), or grade 3 (1) lameness. Hoof tester scores did not differ significantly between groups A and B and were not useful for detecting laminitis or assessing its severity. Onset of laminitis ranged from 24 to 36 hours and from 12 to 36 hours after administration of the induction dose of OF in groups A and B, respectively.

The 3 horses in group A that developed clinical laminitis received 2 doses of flunixin meglumine (1.1 mg/kg, IV) 8 to 12 hours apart and then 2 g of phenylbutazone administered orally every 12 hours on the second day, followed by 1 g of phenylbutazone administered orally every 12 hours until laminitis was no longer detectable via lameness evaluation. Duration of NSAID treatment ranged from 1 to 7 days. Two of the 3 horses in group A that developed clinical laminitis responded positively to NSAID treatment, and no abnormalities were detected via lameness evaluation when phenylbutazone administration was discontinued. These horses were observed for a period of 14 days and were then euthanized because of their preexisting conditions. The other horse that developed laminitis (Obel grade 2) after receiving 5.0 g of OF/kg also recovered after being treated and was returned to the teaching and research herd. Four of the 5 horses in group A that did not develop clinical laminitis were euthanized at the completion of the study; and histopathologic evidence of laminitis was detected in 1 horse that underwent postmortem ex-
Glucose dynamics were altered during the development of laminitis, including multifocal epidermal laminar hyperplasia and keratinocyte apoptosis. The remaining horse in group B developed OF-induced laminitis that was associated with Obel grade 1 lameness and was treated with phenylbutazone. This horse recovered from laminitis and was transferred to the teaching and research herd. Both the horse from group A that developed laminitis and this horse remained in the teaching and research herd for > 6 months, and no lameness problems were observed by farm personnel.

Resting plasma glucose concentrations significantly (P = 0.001) increased in horses that received the OF induction dose (treatment-time effect), but serum insulin concentrations did not differ significantly among groups (Figure 2). A significant (P = 0.002) treatment-time effect was detected for AUCg when FSIGTT data from groups A and C were compared (Figure 3; Table 1). Compared with the mean value at -24 hours, the mean AUCg values for group A horses at the 24- and 48-hour time points were 33.6% and 32.1% higher, respectively. However, the mean AUCg values for group C horses at the 24- and 48-hour time points were 8.7% and 9.1% lower, respectively, than the mean value at -24 hours. Treatment-time effects were not significant for AUCi. With the exception of 1 dataset from an FSIGTT performed in a group C horse at -24 hours, minimal model analyses were successfully performed on all datasets; findings indicated that treatment-time effects were not significant for the minimal model variables. There was no effect of laminitis on any of the variables examined when it was included in the model as a fixed effect.

Discussion

Results of the present study indicated that laminitis can be induced following administration (via nasogastric intubation) of OF at doses of 5.0 or 7.5 g/kg in horses that have received 1 g of OF/kg once daily for 6 days. Some of the horses that developed laminitis recovered clinically, as determined via lameness evaluations. A disturbance in glucose dynamics was detected in the study horses after administration of either laminitis induction dose of OF, and this alteration persisted for < 48 hours. However, no alterations in insulin dynamics were detected after administration of the laminitis induction dose of OF. Glucose dynamics were altered during the developmental phase of laminitis at a time when clinical signs of systemic inflammation were initially detected. All of the horses that received a laminitis induction dose of OF developed combinations of clinical signs that included signs of depression, decreased appetite, diarrhea, fever, tachycardia, and tachypnea. These findings are consistent with those of a previous study in
which horses received induction doses of 7.5, 10.0, and 12.5 g of OF/kg. Oligofructose undergoes minimal digestion within the stomach and small intestine, so most of this carbohydrate enters the cecum and colon where it is used as a substrate by resident bacteria. Lactate production increases within the cecum and colon in response to this alteration, thereby decreasing the intraluminal pH; in turn, this results in a major shift in types of bacterial flora from predominantly gram-negative organisms to predominantly gram-positive bacteria.

Recently, it has been established that streptococci proliferate within the cecum of horses in response to OF administration and that these organisms are likely to have an important role in the development of laminitis.

Four streptococcal isolates have been recovered from the cecum and rectum of horses treated with OF: 2 isolates of *Streptococcus lutetiensis* (previously described as *Streptococcus bovis*) and 2 novel species named *Streptococcus henryi* and *Streptococcus caballi*. However, there is also evidence that endotoxemia develops as a result of carbohydrate overload, and this finding cannot be attributed to the proliferation of gram-positive streptococci. Sprouse et al. detected high blood lipopolysaccharide concentrations during the developmental phase of laminitis induced by starch overload.

Endotoxemia may contribute to the development of laminitis or may simply indicate that intestinal permeability has increased as a result of carbohydrate overload. If present, endotoxemia would be expected to stimulate a systemic inflammatory response that is characterized by anorexia, fever, tachycardia, and tachypnea. These clinical signs were detected in horses treated with a laminitis induction dose of OF in our study. Laminitis itself may be triggered by toxins secreted by streptococci that activate matrix metalloproteinases; the activation of matrix metalloproteinases then leads to selective degradation of basement membrane proteins and failure of the basement membrane–epidermal attachment apparatus.

Destruction of the basement membrane and attachment failure (dysadhesion) is a key process in the development of laminitis, and matrix metalloproteinases may be activated by circulating cytokines or factors released from the large intestine at times when intestinal permeability increases.

In the present study, glucose and insulin dynamics were evaluated by use of FSI GT testing, which is preferred over the euglycemic hyperinsulinemic clamp technique because it is easier to perform and allows assessment of the pancreatic insulin response. Data were analyzed by use of 2 methods—calculation of AUC values and application of the minimal model. The fitting of data to the minimal model increased variability in results between and within horses; thus, treatment-time effects were not significant for minimal model variables. However, resting plasma glucose concentrations increased over time, and mean AUCg values were significantly higher at 24 and 48 hours after induction of laminitis, compared with 0-hour values, in horses that received 5.0 g of OF/kg. The alteration in glucose dynamics occurred when clinical signs of systemic inflammation were detected, before the onset of laminitis. Unfortunately, this alteration in glucose dynamics could not be further characterized on the basis of minimal model results because significant differences were not detected between groups A and C. More horses must be evaluated in future studies to overcome differences in individual animal responses and variability associated with application of the minimal model.

High AUCg values are unlikely to represent an alteration in glucose dynamics induced by OF itself because this carbohydrate is not digested within the small intestine of horses. However, it is conceivable that OF altered volatile fatty acid production within the large in-
testine and that this affected glucose dynamics. A more likely explanation for this alteration in glucose metabolism is that endotoxemia and systemic inflammation developed after OF administration. Horses developed fever before the FSIGTT was performed at the 24-hour time point, which suggests that endotoxins or other bacterial by-products were already entering the circulation from the intestinal tract. In previous experiments, it was determined that insulin sensitivity decreases in response to endotoxemia, and results of another study performed by other researchers support this finding. Tumor necrosis factor-α may mediate the development of IR in horses. Blood in response to endotoxemia, and this cytokine performed by other researchers support this finding. Tumor necrosis factor-α may mediate the development of IR in horses.

**Table 1—** Mean ± SD AUCg, AUCi, SI, Sg, and AIRg values for 8 horses that were administered 5.0 g of OF/kg mixed with 4 L of water (group A) and for 8 horses that were administered 4 L of water alone (group C) via nasogastric intubation (at 9 hours).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time point (h)</th>
<th>Group A</th>
<th>Group C</th>
<th>Treatment-time effect P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCg (× 10^7 mg/dL•min)</td>
<td>–24</td>
<td>27.0 ± 3.9</td>
<td>31.7 ± 8.2</td>
<td>0.002</td>
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<td>6</td>
<td>28.1 ± 7.0</td>
<td>29.0 ± 2.3</td>
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<td></td>
<td>24</td>
<td>36.1 ± 8.6*</td>
<td>28.9 ± 2.4</td>
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<tr>
<td></td>
<td>48</td>
<td>25.7 ± 8.4*</td>
<td>28.8 ± 3.1</td>
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<tr>
<td></td>
<td>72</td>
<td>29.9 ± 3.3</td>
<td>30.1 ± 4.4</td>
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<tr>
<td>AUCi (× 10^7 mU/L•min)</td>
<td>–24</td>
<td>19.0 ± 14.3</td>
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<td>0.4 ± 5.4</td>
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<td></td>
<td>24</td>
<td>15.8 ± 8.2</td>
<td>9.7 ± 4.3</td>
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<td></td>
<td>48</td>
<td>18.8 ± 12.5</td>
<td>10.6 ± 6.6</td>
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<td></td>
<td>72</td>
<td>15.8 ± 11.5</td>
<td>9.8 ± 5.0</td>
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<td>SI (× 10^4 L•min⁻¹•mU⁻¹)</td>
<td>–24</td>
<td>1.85 ± 1.83</td>
<td>1.88 ± 1.38</td>
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<td>6</td>
<td>1.84 ± 1.84</td>
<td>1.20 ± 0.66</td>
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<td></td>
<td>24</td>
<td>0.54 ± 0.52</td>
<td>1.37 ± 0.05</td>
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<td></td>
<td>48</td>
<td>0.54 ± 0.97</td>
<td>1.79 ± 1.62</td>
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<td></td>
<td>72</td>
<td>1.97 ± 2.05</td>
<td>2.13 ± 1.41</td>
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<td>Sg (× 10⁻² min⁻¹)</td>
<td>–24</td>
<td>2.80 ± 1.20</td>
<td>1.61 ± 0.73</td>
<td>0.401</td>
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<td></td>
<td>6</td>
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<td>24</td>
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<td></td>
<td>48</td>
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<td>72</td>
<td>1.70 ± 0.52</td>
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<td>AIRg (mU•min⁻¹)</td>
<td>–24</td>
<td>713 ± 495</td>
<td>628 ± 413</td>
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<tr>
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<td>6</td>
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<td>48</td>
<td>912 ± 435</td>
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<td></td>
<td>72</td>
<td>828 ± 561</td>
<td>832 ± 1,156</td>
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*Within a row, mean value for group A was significantly (P < 0.05) different from the value for group C.*
dependent. Serum insulin concentrations did not differ significantly among groups, but comparisons were hindered by wide variation in baseline values.

Alterations in blood glucose concentrations have previously been detected in horses following experimental induction of laminitis. Galey et al.12 reported that serum glucose concentrations significantly increased over a 12-hour period in horses that were treated with black walnut extract, although the same change was detected in the untreated control group. Horses developed neutropenia in the 4-hour period following black walnut extract administration; clinical signs of laminitis were evident at 8 to 12 hours. In a more recent study,23 significantly higher plasma glucose concentrations and a peak in serum insulin concentrations were detected at 3 to 5 hours after a ration containing 85% cornstarch and 15% wood flour was administered to horses to induce laminitis. These horses were euthanized 8 or 16 hours after administration of the ration, before neutropenia or clinically apparent laminitis developed. However, high plasma glucose and serum insulin concentrations following starch administration may represent postprandial responses to this hydrolysable carbohydrate.24

In the present study, 3 of 8 horses that were treated with the 5.0 g/kg dose of OF developed clinical signs of laminitis, including bounding digital pulses and lameness upon circling, and histologic examination of tissues collected from another horse that was euthanized because of its preexisting condition revealed evidence of laminitis. Thus, half of the horses that were treated with 5.0 g of OF/kg developed laminitis, which establishes that the lower OF dose can be used to experimentally induce laminitis in horses. Results also indicated that horses can survive the laminitis that develops as a result of OF administration. Lameness was not detected 14 days after induction in all 3 horses of group A that developed laminitis. One of these horses and a mare from group B were subsequently returned to the teaching and research herd, and no further lameness was reported by farm personnel over the following 6 months. However, lameness evaluations were not performed by a veterinarian after completion of the study, and the full extent of damage caused by OF-induced laminitis was not determined. Damage was not assessed via radiography or histologic examination of hoof wall biopsy specimens in our study.

Detection of histopathologic evidence of laminar damage in 1 horse that did not develop lameness suggested that subclinical laminitis can develop in OF-treated horses. Structural damage to laminae may develop without detectable clinical signs of laminitis, or individual horses may vary with respect to their pain tolerance. In our clinical practice, we have detected radiographic evidence of third phalanx rotation in horses that do not have a history of lameness according to their owners. Additional studies are required to further evaluate use of the low-dose OF treatment for induction of laminitis in horses and to address several weaknesses of the present study, including the small number of horses evaluated, lack of a standardized NSAID treatment protocol, and absence of histologic examination data for some horses. A protocol for NSAID treatment should be established so that survival rates can be determined for horses with laminitis induced via administration of 5 g of OF/kg, without results being affected by variability in the dosages or duration of drug treatments. Histopathologic data were not available for 3 horses that received the 3 g/kg dose of OF, and this oversight impacts the conclusions drawn from our study. Four of 8 horses that received 5 g of OF/kg developed clinical or histopathologic evidence of laminitis, but laminar damage may have been detected in more of the horses if they had been examined.

Horses were fed 1.0 g of OF/kg/d with oats for 6 days prior to administration of the laminitis induction dose of OF in the present study. This protocol differed from that used by van Eps and Pollitt,2 which involved feeding OF in amounts equivalent to 10% of the induction dose (0.75 to 1.25 g of OF/kg) for 3 days prior to induction of laminitis. Administration of OF in feed at a dosage of 1.0 g/kg/d for 3 days lowers the fecal pH and increases the number of streptococcal bacteria found within the feces of horses.2 A 6-day preinduction feeding period was selected in our study to maximize the response to the lower induction dose of OF. Oats were fed during the same period because van Eps and Pollitt2 reported that horses developed severe hemococoncentration and hypovolemia if they were not acclimated to a higher nonstructural carbohydrate diet.

Use of OF overload to induce laminitis has been criticized because it is questioned whether horses can ingest this amount of fructan when grazing on pasture. However, it has been estimated that horses kept on pasture ingest 3.5 to 7.3 kg of fructan/d when the water-soluble carbohydrate content of the grass peaks at certain times of the year.1 Amounts of OF administered to horses in the present study were within this range—a horse weighing 500 kg received 2.5 kg of OF when the 5.0 g/kg dose was administered. However, fructan consumption by grazing horses often occurs during a 12- to 24-hour period, which is not the same as the bolus treatment administered in our study. The importance of fructans in the development of pasture-associated laminitis must still be determined. It is likely that several fermentable carbohydrates within pasture grasses combine to induce changes in the gastrointestinal tract that induce laminitis in horses.

On the basis of findings of the present study, we conclude that laminitis can be experimentally induced in horses via administration of OF at a dose of 5.0 g/kg and that horses can recover clinically from the OF-induced laminitis that develops. Among the study horses, clinical signs of systemic inflammation were observed following OF administration and glucose dynamics were altered at 24 and 48 hours after the laminitis induction dose of OF was given. In horses grazing on pasture, carbohydrate-overload events that induce intestinal disturbances and precipitate laminitis are likely to occur.

a. Rafifeed OPS, Orafti Active Food Ingredients, Tienen, Belgium.
  b. Dormosedan, Pfizer, Exton, Pa.
  d. Equi-Phar, Vedco Inc, St Joseph, Mo.
  e. ButaTabs E, Butler Animal Health, Dublin, Ohio.
  f. Abbocath-T 14 G X 140 mm, Abbott Laboratories, North Chicago, Ill.

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Butterfly, Abbott Laboratories, North Chicago, Ill.

d. Dextrose, 30% injection, Abbott Laboratories, North Chicago, Ill.

e. Humulin R, Eli Lilly and Co, Indianapolis, Ind.

f. Glucose, Roche Diagnostic Systems Inc, Somerville, NJ.

g. Kobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.

h. Coat-A-Count insulin, Diagnostic Products Corp, Los Angeles, Calif.

i. SAS, version 9.1, SAS Institute Inc, Cary, NC.

j. Provided by Raymond Boston, MinMod Millenium, version 6.10, University of Pennsylvania, Kennett Square, Pa.

k. Stata 9.2, Stata Corp, College Station, Tex.

References


