Effect of diet composition and feeding pattern on the prececal digestibility of starches from diverse botanical origins measured with the mobile nylon bag technique in horses

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ABSTRACT: This trial was conducted to determine the extent of prececal starch digestibility depending on the botanical origin of starch and on diet characteristics (i.e., composition and feeding pattern). The prececal disappearance of six substrates (oats, barley, corn, horse bean, potato, and wheat) was measured in four cannulated horses fed (as-fed basis) 11.8 g/kg BW of a high-fiber (HF) or high-starch (HS) pelleted feed and 10.0 g/kg BW of meadow hay using the mobile nylon bag technique (MBT). The daily feeding pattern was either three meals (two meals of pellets and one meal of hay) or five meals (three meals of pellets and two meals of hay). The experimental procedure was a 2 × 2 factorial arrangement tested in a Latin square design. After 2 wk of adaptation to the diet, collections were made on 5 d. Thirty nylon bags, composed of five bags of each substrate, were intubated to each horse during the ingestion of the morning meal. Bags were collected in the cecum, using a magnet, at 9 h postintubation. In spite of strong interindividual differences, approximately 80% of the intubated bags were collected. On average, the mean retention time of the bags was 6.2 h (± 0.17).

Regardless of the feeding pattern, the transit of the bags was faster when the fiber content of the diet was higher (P = 0.003). Likewise, regardless of the meal composition, transit was also faster when the ration was split into five daily meals (P = 0.001). The DM disappearance, corrected with particulate losses (DMDc), differed depending on the substrate tested (33.5, 57.1, 63.8, 67.7, 78.6, and 86.2% for potato, horse bean, oats, barley, corn, and wheat, respectively; P = 0.001). The DMDc of corn, barley, and potato was higher when HS was fed (P = 0.020); regardless of the substrate, DMDc was higher with five daily meals (P = 0.001). The starch disappearance (StarchDc) was different depending on the substrate (P = 0.001; 36.1, 71.2, 86.6, 89.2, 99.0, and 99.7% for potato, horse bean, barley, corn, wheat, and oats, respectively). Whatever the substrate, StarchDc was higher when HS was fed (P = 0.007), but it was not affected by the feeding pattern of the diet. Although passage rate was modified and feed intake was different, the botanical origin of starch was the main factor that affected prececal starch disappearance in horses.

Key Words: Foregut, Horse, Mobile Nylon Bag, Nutrition Physiology, Starch Digestion, Transit Time


Introduction

The starch stored in grains represents an important energy source used to meet the athletic horse’s energy requirements. However, the inclusion of starch in the diet needs much attention. Feeding more than 4 g of starch/kg BW at a time was shown to lead to the overload of small intestinal digestive capacity (Potter et al., 1992) and to nondegraded starch reaching the large intestine. As a consequence, the energy yield of the diet is reduced, and intestinal disorders may appear due to microbial profile disturbance. Therefore, feeding less than 2 g/kg BW of starch per meal is usually recommended (Meyer et al., 1995). Studies conducted with ileum-fistulated horses have shown that the extent of prececal starch digestion is related to numerous factors interacting together: starch intake, its processing, its botanical origin, and the closeness of forage distribution. However, keeping ileally cannulated horses in good health is difficult, and starch sources can only be tested one after the other, which is time consuming. In addition, due to the variable starch content of feedstuffs, it is impossible to maintain a similar starch intake at a similar level of DMI. Therefore, it is hard to isolate the effect of the botanical origin of starch on...
available data. Consequently, the use of mobile bags to estimate prececal starch digestibility, with cecal-fistulated horses, represents an alternative method, as it allows one to compare different feedstuffs simultaneously.

The mobile bag technique (MBT) was set to estimate the prececal disappearance of different starch sources depending on meal characteristics and forage distribution: two distinct pelleted feeds and two feeding patterns were chosen according to French feeding practices in stables. In addition, the MBT was used to assess the influence of diet characteristics on prececal transit of solid digesta.

Materials and Methods

Experimental Design and Treatments

Four crossbred mature geldings (mean age = 11 yr; mean BW = 428 ± 32 kg), each fitted with a polyvinyl chloride cannula (i.d. 22.5 mm) in the cecum, were used. Animals were surgically prepared by a certified large-animal veterinarian, using the technique described by Drogoul et al. (2000), at least 1 yr before the trial. The project was conducted in the ENESAD facilities (Tart-le-Bas, Côte d’Or, France) under license from the Department of Health and Animal Care of the French Veterinary authority (A 21 622 007). Horses were turned out every other day in a round pen where they were led at a steady trot for 30 min.

Preparation, Intubation, and Collection of Bags

Mobile bags (6 cm × 1 cm diameter) were prepared from monofilament nylon (Nylon blutex, SAATI France, Sailly Saillesel, France; 46 μm porosity) and heat sealed (M171 232 00 type Uh 32, Thimonnier SA, Lyon, France); extremities were folded to prevent any sharp and possibly irritating ends; to allow bag capture, a steel washer (1 cm external diameter) was sealed into one end of each bag (Moore-Colyer et al., 1997). Bags were filled with 400 mg of substrate ground to pass a 3-mm mesh. Six substrates of diverse botanical origins were tested: barley (Hordeum vulgare), corn (Zea mays), horse bean (Vicia faba minor), oats (Avena sativa), wheat (Triticum durum), and freeze-dried raw potato (Solanum tuberosum). Potato, which is not a usual constituent of horse diet, was chosen as a reference of resistant starch. The chemical composition, as well as the particle size distribution (laser light diffraction in dry conditions) of the substrates, was determined (Table 3).
Table 2. Daily feeding patterns of diet tested

<table>
<thead>
<tr>
<th>Time</th>
<th>Five meals</th>
<th>Three meals</th>
</tr>
</thead>
<tbody>
<tr>
<td>0800</td>
<td>3.93 g/kg BW of pellets</td>
<td>7.50 g/kg BW of pellets</td>
</tr>
<tr>
<td>1000</td>
<td>5.00 g/kg BW of meadow hay</td>
<td>—</td>
</tr>
<tr>
<td>1200</td>
<td>3.93 g/kg BW of pellets</td>
<td>10.00 g/kg BW of meadow hay</td>
</tr>
<tr>
<td>1600</td>
<td>5.00 g/kg BW of meadow hay</td>
<td>—</td>
</tr>
<tr>
<td>1800</td>
<td>3.93 g/kg BW of pellets</td>
<td>4.30 g/kg BW of pellets</td>
</tr>
</tbody>
</table>

*aQuantity fed (g/kg BW) on an as-fed basis.

Mobile bags were intubated right after the ingestion of a third of the morning pelleted meal. Bags were washed out in the esophagus through a nasogastric tube (1.8 cm external diameter; 150 cm long) with 750 mL of tap water. The remainder of the meal was fed right after the intubation, and meal consumption was checked to ensure that the intubation did not cause any trouble with intake. A polyester bag, containing a magnet (72 × 15 × 10 mm), was introduced inside the cecum through the cannula, to place the magnet near the ileocecal junction (Moore-Colyer et al., 1997). This device allowed bag capture right at the bag’s arrival in the cecum. The magnet was withdrawn every 0.5 h, starting 2 h after intubation, until the distribution of the last pelleted meal of the day. Bag collection occurred as follows: after opening the cannula, the magnet was pulled out of the cecum and then reintroduced inside the cecum and pulled out again. If no bag were collected, the magnet was replaced in the cecum until no more bags were caught. This allowed us to collect bags that would eventually be lost as the magnet passed through the cannula. Collected bags were rinsed rapidly under tap water then stored at −20°C.

For each horse and each intubation, a batch of 30 bags composed of five of each substrate tested was prepared and intubated to each horse on each day of collection.

Treatment of Bags and Measurements

For each day of collection throughout each experimental period, the proportion of bags collected was calculated (BC). In addition, the characteristics of the transit of the mobile bags was assessed through the analysis of the curves representing the cumulated percentage of bags as a function of time, and through the calculation of the mean retention time (MRT) of the bags in the prececal part of the digestive tract according to Faichney (1975):

\[
\text{MRT} = \frac{\sum B_i \times \Delta t_i}{\sum B_i}
\]

where \(B_i\) = number of bags collected at time \(t_i\) and \(\Delta t_i\) = average time spent since \(t_0\) = time of bags intubation: \(\Delta t_i = (t_i - t_0) + [(t_i - t_0)/2]\).

At the end of each experimental period, all bags were thawed at room temperature, and then washed in a domestic washing machine (Alternatic program, Calor, Ecully, France) with demineralized water (two to three cycles of 5 min) until rinsing water was clear. Bags were then sorted and dried in a forced-air oven at 60°C to constant weight. Each bag was weighed to calculate the DM disappearance (DMD). Bags containing a com-

Table 3. Chemical composition (DM basis) and proportion of particles smaller than 48 μm of starch sources (ground at 3 mm) tested in mobile bags

<table>
<thead>
<tr>
<th>Composition, %</th>
<th>Oats</th>
<th>Wheat</th>
<th>Horse bean</th>
<th>Corn</th>
<th>Barley</th>
<th>Potato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>37.0</td>
<td>61.5</td>
<td>37.3</td>
<td>67.4</td>
<td>51.9</td>
<td>67.1</td>
</tr>
<tr>
<td>CP</td>
<td>8.3</td>
<td>10.5</td>
<td>28.9</td>
<td>8.7</td>
<td>10.4</td>
<td>10.1</td>
</tr>
<tr>
<td>Ash</td>
<td>2.4</td>
<td>1.5</td>
<td>3.6</td>
<td>1.5</td>
<td>2.5</td>
<td>4.2</td>
</tr>
<tr>
<td>NDF</td>
<td>37.9</td>
<td>14.7</td>
<td>19.8</td>
<td>12.1</td>
<td>23.2</td>
<td>9.4</td>
</tr>
<tr>
<td>ADF</td>
<td>17.9</td>
<td>3.0</td>
<td>10.4</td>
<td>3.0</td>
<td>6.7</td>
<td>2.1</td>
</tr>
<tr>
<td>ADL</td>
<td>3.9</td>
<td>0.9</td>
<td>1.4</td>
<td>0.7</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Particle size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;48 μm, %</td>
<td>6.9</td>
<td>6.9</td>
<td>12.4</td>
<td>9.3</td>
<td>7.1</td>
<td>20.1</td>
</tr>
</tbody>
</table>

*aDiameter of the theoretical sphere determined with laser light diffraction in dry conditions according to Melcion (2000).
mon substrate and collected the same day for a common horse were pooled and substrate residuals were ground together using a ball mill (Mixer mill MM 200, Retsch, Haan, Germany), after which a representative sample of the experimental period was made by mixing an aliquot of ground residues of each day. Residual starch was analyzed by an enzymatic procedure (modified by Kozlowski, 1994) and starch disappearance (StarchD) was calculated.

**Loss of Particles from Mobile Bags**

Two different procedures were used to determine the loss of particles from mobile bags. The first one (incubation) was adapted from Philippeau and Michalet-Doreau (1997) and consisted in incubating bags in a medium formulated to mimic the physicochemical characteristics of gastric content of horses during the meal. The incubation method was chosen to determine the losses occurring through the pores of the nylon immediately after intubation. The second procedure (washing) was performed to assess the additional losses of particles occurring during the rinsing procedure (cold-water mechanical washing). In the incubation procedure, five nylon bags, identical to those previously described, were placed into five bottles containing approximately 50 mL of a moderately acidic (pH 5.5) medium and composed of 80% artificial equine saliva (for 1 Lo f solution: 984.8 mL of H2O; 15.2 mL of 3 N HCl; 6.7156 g of Na2CO3; 6.0164 g of NaHCO3; 5.7137 g of CaCl2·2H2O; 3.0615 g of MgCl2·6H2O; 2.2743 g of KCl; 1.3361 g of Na2HPO4·12 H2O; 1.1011 g of NaCl) and 20% 0.625 M hydrochloric acid, chosen to mimic gastric juice. After a 1-h incubation in a shaking water bath at 38°C, bags were withdrawn from the bottles and rapidly rinsed with demineralized water. In the washing procedure, 10 nylon bags of each substrate were immersed in distilled water for 1 h, and then washed in a domestic washing machine as described earlier. All bags were dried at 60°C to constant weight. Each bag was weighed to determine the fraction lost during incubation and the corrected DMD (DMDc) was calculated. Likewise, StarchD were corrected and the corrected starch disappearance calculated (StarchDc).

The DMD from mobile bags collected from the cecum were then corrected for each bag excluding the fraction lost during incubation and the corrected DMD (DMDp) was calculated. Likewise, StarchD were corrected and the corrected starch disappearance calculated (StarchDp).

**Statistical Analyses**

An ANOVA with the GLM procedure of SAS (version 8.2; SAS Inst. Inc., Cary, NC) was done to evaluate the response of MRT to the pellet composition (Pc), to the diet feeding pattern (F), and to the Pc × F interaction. The period, the day of intubation (nested in the period), and the horse, which was specified as a randomized effect, were also included in the model. In addition, BC was included in the model as a covariate. A repeated-measures ANOVA was performed to compare the profiles of cumulated bag collection using the repeated time option. The effect of bag collection time was found to be significant; therefore, bag collection data were analyzed individually for each collection time in the bar chart.

Another ANOVA was used to evaluate the effect of the starch source on the loss of particles from nylon bags (DMDp and StarchDp). Losses were analyzed within each method using the “by” option. A last ANOVA was performed to evaluate the variables’ response (DMDc and StarchDc) to the main effects: substrate tested in the bag (S), Pc, and F; the interactions S × Pc, S × F, and Pc × F were also tested in the model. The period, the day of intubation (nested in the period), and the horse, which was specified as a randomized effect, were included in the model (the day of intubation was removed from the model for StarchDc analysis as the constitution of the representative samples for each period masked day effect). Least squares means were calculated for all variables and separated using pairwise t-tests (PDIF option of SAS) when the main effect of the response variable was significant at the significance threshold of 0.05, which was used for all tests.

**Results**

On average, 79.8% (± 2.5) of the intubated bags were collected in the cecum during the 9-h collection period. The average of BC was different between horses, ranging from 58.1 (± 6.5) to 92.3% (± 2.3).

Regardless of diet characteristics and horses, less than 1% of the intubated bags were collected 2.0 h after intubation; on average, the first appearance of bags in the cecum occurred between 2.5 and 3.0 h after intubation.

The kinetics of cumulative BC were different depending on pellet composition and the number of meals per day (Figure 1). The BC started earlier when pellet HF was included in the diet: 2.5 h after intubation, BC was greater with HF compared with HS (P = 0.039). For each collection performed after 2.5 h postintubation and regardless of the number of meals offered, BC was higher when pellet HF was fed rather than HS (P = 0.001 to 0.015). In addition, for each collection performed between 4.0 h and 8.0 h postintubation, whatever the pellet composition, BC was greater when the daily ration was split into five meals instead of three meals (P = 0.001 to 0.049). However, for collections occurring after 3 h postintubation, the kinetic of BC was similar when horses were given the combination HF-three meals and when they were fed HS-five meals. At each collection performed after 2.5 h postintubation, BC was higher when horses were offered the combination HF-three meals rather than HS-three meals (P = 0.001 to 0.042).

On average, the mean retention time of the bags in the prececal part of the digestive tract was 6.2 h (±
Figure 1. Cumulative collection of mobile nylon bag in the cecum of horses fed a diet composed either of a high-fiber (HF) or a high-starch (HS) pellet and hay, in three (two meals of pellets + one meal of hay) or five (three meals of pellets + two meals of hay) daily meals. a,b,cWithin a time of collection, bars without a common superscript letter differ, $P = 0.05$.

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0.17). Whatever the diet characteristics, the MRT of the bags was different between horses (from 5.5 to 7.3 h; $P = 0.001$); the MRT was shorter when HF was included in the diet (5.9 vs. 6.6 h, for HF and HS, respectively; $P = 0.003$), and it was also shorter when horses received five meals per day (5.9 vs. 6.6 h, for five meals and three meals, respectively; $P = 0.001$); the interaction between the type of pellet and the feeding pattern was not significant (Table 4).

The mean loss of DM after bag incubation in artificial gastric content was 8.3% ($\pm 0.72$; $n = 39$). The DMD$_D$ was different depending on the substrates tested ($P = 0.001$). The greatest loss was observed for potatoes (15.0%), whereas the smallest loss was noted for oats and corn (4.3%; $P = 0.001$; Table 5). The associated starch loss was also strongly related to the type of substrate (mean StarchD$_D$ = 3.8% ± 0.69; $n = 39$; $P = 0.001$; Table 5). Consequently, compared with total DM loss, the starch content of the particulate fraction was 0.0, 50.2, 13.1, 100.0, 11.7, and 24.1% for oats, wheat, horse bean, corn, barley, and potato, respectively.

On average, the loss of DM after washing the bags was 22.4% ($\pm 0.89$; $n = 25$). The DMD$_c$ was different depending on the substrate tested ($P = 0.001$). The greatest loss was measured in bags containing oats (30.4%), whereas the smallest ones were observed for wheat and barley (19.3%; $P = 0.001$; Table 5). The starch loss induced varied greatly between starch sources (mean StarchD$_c$ = 23.7 ± 1.78%; $n = 25$): from 14.1% for potato to 42.0% for oats ($P = 0.001$) (Table 5). However, except for oats (30.8%), the starch content of the substrate remaining in the bags was fairly close to the starch content of the substrate initially introduced: 59.1, 37.9, 65.9, 51.2, and 73.0% for wheat, horse bean, corn, barley, and potato, respectively.

The DMD$_c$ from the bags collected in the cecum was affected by the substrate tested ($P = 0.001$). Regardless of pellet composition or feeding pattern, DMD$_c$ was 33.5, 57.1, 63.8, 67.7, 78.6, and 86.2%, for potato, horse bean, oats, barley, corn, and wheat, respectively; all pairwise comparisons between substrates were significant ($P = 0.001$). Regardless of the substrate, DMD$_c$ was higher when HS was included in the diet (63.8 vs. 65.2% for HF and HS, respectively; $P = 0.001$), and also when the daily ration was split into five meals (63.9 vs. 65.0% for three meals and five meals, respectively; $P = 0.001$). The interaction between pellet composition and the type of substrate was significant ($P = 0.001$), but other tested interactions were not. The DMD$_c$ of oats, wheat, and horse bean were similar between the two pelleted feeds.

### Table 4. Mean retention time (MRT) of bags in the prececal part of the digestive tract in horses fed a high-fiber (HF) or a high-starch (HS) pellet, and hay, in three or five daily meals

<table>
<thead>
<tr>
<th>Diet</th>
<th>HF-three meals</th>
<th>HF-five meals</th>
<th>HS-three meals</th>
<th>HS-five meals</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRT, h</td>
<td>6.2$^{a}$</td>
<td>5.6$^{a}$</td>
<td>7.0$^{c}$</td>
<td>6.2$^{b}$</td>
<td>0.9</td>
</tr>
</tbody>
</table>

$^{a,b,c}$Least squares means ($n = 74$) without a common superscript letter differ, $P = 0.05$. 
Table 5. Dry matter (DMD<sub>p</sub>) and starch (StarchD<sub>p</sub>) disappearance (mean ± SD) from mobile bags for each starch source tested after incubation or washing<sup>a</sup>

<table>
<thead>
<tr>
<th>Starch source</th>
<th>Method</th>
<th>Oats</th>
<th>Wheat</th>
<th>Horse bean</th>
<th>Corn</th>
<th>Barley</th>
<th>Potato</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation (n = 39)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMD&lt;sub&gt;p&lt;/sub&gt;</td>
<td>4.3&lt;sup&gt;y&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;y&lt;/sup&gt;</td>
<td>12.7&lt;sup&gt;y&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;x&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;x&lt;/sup&gt;</td>
<td>15.0&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>StarchD&lt;sub&gt;p&lt;/sub&gt;</td>
<td>-5.5&lt;sup&gt;y&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;y&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;y&lt;/sup&gt;</td>
<td>8.9&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;x&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;x&lt;/sup&gt;</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Washing (n = 25)</td>
<td>30.4&lt;sup&gt;y&lt;/sup&gt;</td>
<td>19.3&lt;sup&gt;y&lt;/sup&gt;</td>
<td>24.9&lt;sup&gt;y&lt;/sup&gt;</td>
<td>20.5&lt;sup&gt;y&lt;/sup&gt;</td>
<td>19.3&lt;sup&gt;y&lt;/sup&gt;</td>
<td>21.0&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>DMD&lt;sub&gt;p&lt;/sub&gt;</td>
<td>42.0&lt;sup&gt;y&lt;/sup&gt;</td>
<td>20.5&lt;sup&gt;y&lt;/sup&gt;</td>
<td>23.6&lt;sup&gt;y&lt;/sup&gt;</td>
<td>22.2&lt;sup&gt;y&lt;/sup&gt;</td>
<td>20.3&lt;sup&gt;y&lt;/sup&gt;</td>
<td>14.1&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>A 1-h incubation in artificial gastric content at pH 5.5 or three consecutive washings in demineralized water in a domestic washing machine.

<sup>y,x</sup> Least squares means within an item and a line, without a common superscript letter differ, P = 0.05.

Discussion

We were able to isolate the effect of the botanical origin of starch among numerous parameters affecting its prececal digestibility. The results we obtained using the MBT provided original information regarding the characteristics of the transit of the solid phase of digesta in the foregut of the horse, depending on the morning meal composition and volume.

Our bag collection, reaching approximately 80% of intubated bags (range from 58 to 92%), was similar to that of 82% we observed previously (de Fombelle et al., 2001) and was above those commonly reported: 65% (de Fombelle, 2000); 50 to 75% (Moore-Colyer et al., 1997); and 55% (Macheboeuf et al., 1996). These differences could be related to the characteristics of the diet given to horses, as discussed below. Indeed, the transit time of the bags varied depending on diet composition and feeding pattern; therefore, the amount of bags recovered in the cecum can be different for a same collection period duration. Besides, bag capture in the cecum can be affected by the method used. Similar to Moore-Colyer et al. (1997), we recovered the bags in the cecum thanks to a magnet, whereas the bags used by Macheboeuf et al. (1996) were proceeding manually. The use of a magnet seemed to be more reliable and, indeed, when the magnet was left in the cecum over a 24-h period, the BC reached 100%. Although an unlimited collection period would have allowed for a full bag recovery, bags reaching the cecum after 9-h postintubation were not collected because they were not representative of previous tested, whereas results for corn, barley, and freeze-dried potato were higher when HS was fed (P = 0.001 to 0.020; Table 6).

The StarchD<sub>c</sub> from the bags collected in the cecum was different depending on the substrate tested (P = 0.001). Regardless of the substrate, StarchD<sub>c</sub> was also higher when HS was fed (79.8 vs. 80.8% for HF and HS, respectively; P = 0.007). Other effects tested in the model were not significant. Whatever the characteristics of the diet, oats and wheat starch were the most degraded (99.7 and 99.0%, respectively), and the lowest value was obtained with potato (36.1%). The StarchD<sub>c</sub> for barley, corn, and horse bean was 86.6, 89.2, and 71.2%, respectively. All pairwise comparisons between substrates were significant (P = 0.001), except between oats and wheat (P = 0.211).

Table 6. Prececal DM disappearance (DMD<sub>c</sub>) and starch disappearance (StarchD<sub>c</sub>) corrected for losses that occurred during incubation of substrate in artificial gastric content from substrates tested with the mobile bag technique in horses fed a high-fiber (HF) or high-starch (HS) pellet

<table>
<thead>
<tr>
<th>Starch source</th>
<th>Item</th>
<th>Oats</th>
<th>Wheat</th>
<th>Horse bean</th>
<th>Corn</th>
<th>Barley</th>
<th>Potato</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMD&lt;sub&gt;c&lt;/sub&gt; (n = 1,768)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>63.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>85.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>57.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.7&lt;sup&gt;y&lt;/sup&gt;</td>
<td>66.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.3&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>63.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>86.6&lt;sup&gt;f&lt;/sup&gt;</td>
<td>57.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.5&lt;sup&gt;x&lt;/sup&gt;</td>
<td>68.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>34.6&lt;sup&gt;x&lt;/sup&gt;</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>StarchD&lt;sub&gt;c&lt;/sub&gt; (n = 435)</td>
<td>99.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.0&lt;sup&gt;y&lt;/sup&gt;</td>
<td>85.9&lt;sup&gt;y&lt;/sup&gt;</td>
<td>35.2&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>99.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.5&lt;sup&gt;x&lt;/sup&gt;</td>
<td>87.4&lt;sup&gt;y&lt;/sup&gt;</td>
<td>37.1&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c,d,e,f</sup> Least squares means within an item and a line, without a common superscript letter differ, P = 0.05.

<sup>y</sup> Least squares means within an item and a column without a common superscript letter differ, P = 0.05.
MRT data, as their transit might not have been related to the pelleted meal ingested when bags were intubated. Using those bags for DMD and StarchD would have led to overestimate the results.

Regardless of the horse and the diet, the shortest interval between intubation and bag collection was 2 h, which is longer than previous data reporting intervals between 1 and 2 h (Macheboeuf et al., 1995, 1996; McLean et al., 1999). The increase might be related to the type of the animal used; McLean et al. (1999) worked with ponies (270 kg BW), whereas we used horses (400 kg BW). As mentioned above, the effect of the diet might also interfere with the time of first appearance in the cecum.

The average MRT of nylon bags in the foregut was 6.2 h, which was higher than the 4.2 h presented by Macheboeuf et al. (1995), but which ranged in the transit time values reported for labeled feeds particles (6.8 ± 1.2 h; Medina et al., 2002). The literature regarding prececal transit in horses is scarce, and most of prececal MRT data were calculated by subtracting the cecal-anal MRT to the oral-anal MRT (Medina et al., 2002). However, because our data are in accordance with previous measurements regarding the transit of particles, we hypothesize that nylon bags behaved like the solid phase of digesta.

Our data clearly demonstrated that the MRT of the bags was modified with both the pellet composition and the feeding pattern of the diet. Regardless of the feeding pattern, the MRT was significantly shorter when HF was fed (5.9 vs. 6.6 h, for HF and HS, respectively). The DMI being identical on a daily basis, this was probably due to the NDF content of HF (40.4 vs. 22.9%, for HF and HS, respectively), as a higher proportion of plant cell walls induces a shorter transit time (Cuddeford et al., 1995; Yoder et al., 1997; Medina, 2003). Our observation confirmed recent data indicating a quicker gastric emptying when a HF diet was fed compared with a HS diet given at the same level of intake (Métyayer et al., 2004). The MRT was also shortened when the daily ration was split into five meals compared with three meals (5.9 vs. 6.6 h, respectively). When the daily ration was split into five meals, the volume of the morning meal was twice smaller than with three daily meals. Smaller meal size has been associated with quicker gastric emptying in terms of percent of total original volume (Métyayer et al., 2004). In addition, feeding hay 2 h after the morning meal in the five meals pattern probably accelerated the prececal transit of feeds, as shown when the forage is fed after a concentrate meal (Muuss et al., 1982; Tisserand, 1992). We also noted that the variation of hay consumption was directly related to interindividual variation of MRT. Horses that would ingest their hay rapidly and had small refusals had a lower MRT. Compared with the “fastest” horse (average MRT = 5.2 h), the “slowest” one (average MRT = 7.8 h) had an opposite eating behavior: this horse was not only more stressed by the intubation, demonstrated by his slower than usual feed ingestion, but his average hay consumption was the smallest of the four horses used in this trial, regardless of the diet tested (data not shown). The case of this horse was in accordance with bibliographic data showing that MRT increases as DMI decreases (Yoder et al., 1997; Pagan et al., 1998; Drogoul et al., 2001). Therefore, we noticed that BC was directly related to hay consumption; it started earlier when the hay meal was close to the morning pelleted meal (i.e., when horses were fed five meals per day). In addition, no bags were recovered from the cecum as long as hay ingestion had not started.

Like every in sacco method, the first limit of MBT use for measuring the prececal digestibility of feedstuffs is the loss of particles through the pores of the nylon. This disappearance of substrate occurs independently of digestion processes and might lead to the overestimation of in vivo digestibility. Losses of the substrate and the particles smaller than nylon porosity occur right at the immersion of the bags in the gastric content. Grinding and substrate characteristics (humidity, hardness, structural defects, etc.) affect the size distribution of particles and thereby the proportion of losses; a linear relationship between DM losses and the proportion of particles smaller than 50 μm was shown (Michalet-Doreau and Cerneau, 1992). However, the lost proportion was smaller than the fraction of fine particles, and it was suggested that a clogging of pores (Lindberg, 1981) and a retention of small particles in a network of larger ones appeared. Our results confirmed that the proportion of DM that was lost during the incubation of bags in artificial gastric content was close to (wheat, horse bean, and barley) or lower than (oats, corn, and potato) the proportion of particles smaller than 48 μm. The biochemical characteristics of the substrate vary among particle size distribution (Melcion, 2000). As evidence, we measured no starch in the fraction that escaped for oats, whereas for corn, the total amount of DM loss was composed entirely of starch. Apart from corn, the proportion of starch in the lost fraction was smaller than in the original substrate. According to ruminant in situ studies (Vanzant et al., 1998), it is hypothesized that the proportion of substrate lost was completely degraded. This assumption can be sustained by the high concentration of bacteria in the stomach of the horse (de Fombelle et al., 2003b). Considering DM loss from mobile bags, the correction of our results might have led us to underestimate the true DMD (−1.1, −1.2, −2.4, −3.1, and −23.0% for wheat, corn, oats, barley, horse bean, and potato, respectively). However, this underestimation was negligible, except for potato. Focusing on starch, it is likely that the proportion that escaped was completely degraded in the stomach of the horse as this compartment sheds numerous starch-utilizing bacteria (i.e., lactobacilli and streptococci; de Fombelle et al., 2003b). Also, it has been recently reported that the apparent digestibility of starch in the stomach of horses can vary from 24 to 98% (de Fombelle et al., 2003a; Varloud et al., 2003). Still, if StarchD-loss were neglected, the overestimation of StarchD would
have been small (0.0, 0.1, 0.2, 1.1, 1.8, and 8.2% for oats, wheat, barley, corn, horse bean, and potato, respectively). Hence, it is likely that these errors can be neglected.

Additional losses occur during the cold-water rinsing procedure of the bags collected from the cecum, a procedure designed to remove mucous, endogenous enzymes and microbial biomass from the feed residue (Van Straalen et al., 1993). Measuring these losses exactly is impossible; however, rinsing bags containing the original substrates allowed us to qualify the losses. The DM losses ranged between 18.2 and 29.1%, indicating that the proportion of escaped particles was far more than the proportion of particles smaller than 48 μm. This greater loss of particles might be mostly due to distortion of pores and weakening of seams (Vanzant et al., 1998). Assuming that the same proportion of the residual substrate was lost through the rinsing procedure, the additional DM loss represented 14.3, 2.9, 11.9, 5.4, 6.7, and 15.0% of the DM initially introduced in the bags for oats, wheat, horse bean, corn, barley, and potato, respectively. Therefore, the rinsing procedure led to an overestimation of DMD data that was negligible (<7%) for wheat, corn, and barley. Except for oats, the proportion of starch measured in the substrate remaining in the bag was very close to that initially introduced. Consequently, we hypothesized that the StarchD measured in the nylon bags collected from the cecum might be qualitatively identical to the true StarchD from the bags. When the starch lost through the rinsing procedure was used to calculate the amount of remaining starch in the bags before rinsing, it appeared that 0.3% of additional starch losses occurred for oats and wheat, 8.6% for horse bean, 2.9% for corn, 3.5% for barley, and 10.4% for potato. Therefore, particulate losses occurring during the rinsing procedure shall not prevent the use of the MBT to measure prececal digestibility of starch in conventional feeds for horses (i.e., cereals). However, further work using ileal-cannulated horses should be conducted to validate these in sacco measurements; besides, as microbial digestion of starch might occur in the cecum, the collection method should be adjusted to suppress the retention time of nylon bags in this compartment.

We fed two pelleted feed of different composition (NDF:starch ratio for HF = 1.82 vs. HS = 0.55) and used two feeding patterns, which modified the prececal retention time. We hypothesized that the feed composition would interfere on the activity of both prececal microorganisms (Kienzle et al., 1997) and endogenous α-amylase (Radicke et al., 1991, 1992; Kienzle et al., 1994). We also hypothesized that the exposure time to these digestive agents would affect the extent of prececal starch digestion (McLean et al., 1999). However, only the feed composition affected StarchD and, regardless of the substrate placed in the bags, a significant 1.0% increase was reported between HF and HS. As the effect of the feeding pattern was not significant, our results suggested that the prececal StarchD was more affected by the composition of the feed itself than by the starch intake although it was 3.7-fold greater in HS-three meals than in HF-five meals. However, the review of bibliographic data showed a decrease in the starch digestibility of oats, corn, and sorghum, regardless of the physical form, when different assays testing an increasing amount of starch intake were compared (Householder, 1978; Kienzle et al., 1992; Meyer et al., 1995). This decrease could be identified even within comparable starch sources (i.e., when a same mechanical or hydrothermal treatment had been applied). As among mechanical treatments, grinding provides the highest starch digestibility. It is likely that grinding our substrates contributed to attenuate the effect of the starch level of intake in our trial.

The DMD of oats, wheat, and horse bean was not affected by the feed composition or by the feeding pattern of the diet. We assumed that the degradability of DM from these three substrates had reached a plateau that was dependent on intrinsic properties and not influenced by feeding practices. The DMD of corn, barley, and potato was higher with HS than with HF. The greatest modification was a 3.8 increase observed for corn. Besides, the effect of the feed composition was toward a moderate increase of DMD with the HS diet, probably due to the longer retention time of the bags when HS was fed. Nevertheless, DMD was higher when the diet was split into five daily meals, although this feeding pattern had the shortest bags retention time. As we measured no relationship between DMD and the time of bag collection, it is unlikely that an extended exposure time enhanced substrate digestibility. It is most probable that the composition of the HS diet stimulated digestive phenomena occurring in the prececal tract. Regardless of the diet, the lowest DMD were measured for potato and horse bean (33.5 and 57.1%, respectively). The DMD of cereals ranged from 63.8% for oats to 86.2% for wheat, negatively correlated to the NDF content. Oats and barley DMD was almost identical to previous data (64.7 and 64.0%, respectively; de Fombelle et al., 2001). The DMD of corn was almost 20 points greater than the data reported by de Fombelle et al. (2001; 78.6 vs. 58.1%, respectively), although horses were fed a diet similar to HS-three meals.

Potato StarchD was the smallest (36.1%), although higher when compared with the 10% apparent digestibility measured for raw potato on fistulated horses (Meyer et al., 1995). Due to the large loss of small particles (20%), our results for this substrate were overestimated. Also, we used freeze-dried grounded potato, enhancing the surface exposed to digestive agents compared with raw potato. All other starch sources, among common feedstuffs fed to horses, had a StarchD above 70%. Horse bean had the lowest StarchD (71.2%). This substrate is usually chosen to increase the proportion of nitrogen in the diet, as shown by its CP content (28.9%); consequently horse bean is not used on its own. As 30% of horse bean starch remained undegraded at the entrance of the cecum, we would recommend keep-
ing horse bean at a low level in the diet to limit the amount of starch delivered to hindgut microorganisms. However, horse bean starch probably does not represent the greatest threat to the hindgut ecosystem, as it was shown to be slowly fermented by ruminal microorganisms (Nocek and Tamminga, 1991). Barley StarchD (86.6%) was close to our previous measurement (81.4%; de Fombelle et al., 2001) and between the values reported on cannulated ponies by Meyer et al. (1995) and Arnold (1982) (30 and 96.9%, respectively). The lowest digestibility might be related to grain processing, as Meyer et al. (1995) used rolled barley instead of ground or cracked barley. In addition, these researchers used ponies fistulated at the end of the jejunum, so their results might be somewhat underestimated. Surprisingly, corn StarchD was close to 90%, although many trials, which tested ground corn at a starch level of intake above 200 mg/100 kg BW, showed a smaller precaecal digestibility: 66.2 (de Fombelle et al., 2001), 45.6 (Meyer et al., 1993), 55.0 (Meyer et al., 1995), and 70.6% (Kienzle et al., 1992). Corn structural characteristics do not facilitate enzymatic hydrolysis and microbial degradation (Kienzle et al., 1997). Recent work conducted in ruminants underlined that cornstarch degradation was affected both by starch composition (amylose:amylopectin ratio) and endosperm texture (dent or flint). Whatever the amylopectin content, cornstarch was better degraded when the endosperm surrounding the starch granule was floury (dent) instead of vitreous (flint; Philippeau et al., 1998). The higher zein content of the vitreous endosperm and of the flint grain is in relation with a higher content of protein storage bodies, which limit the accessibility of starch granules to rumen microorganisms (Michalet-Doreau and Doreau, 1999). This assessment could be true for enzymatic hydrolysis. It can be hypothesized that the corn tested in study was probably more floury corn compared with others. However, this remains to be proven, and further research is required to determine the effect of corn variety differences on precaecal starch digestibility in order to improve and secure its use in horse feeding. Oats and wheat starch had the highest precaecal digestibility (above 99%). These results agree with previous measurements and showed that the starch stored in these cereals can be safely fed to horses, as only a very limited amount will reach the large intestine.

Implications

This trial showed that the mobile bag technique could be used as an alternative tool to estimate in vivo precaecal digestibility of starch sources in horse diets. Despite varying passage rates and levels of starch intake, starch digestibility was mainly related to its botanical origin. As oats and wheat starch were almost entirely digested before reaching the large intestine, we assume that these cereals can be safely used in horse feeding at this level of intake. However, because these feeds are highly fermentable, both their energy use and their influence on the precaecal ecosystem need to be further explored. Corn, barley, and horse bean should be used cautiously because some of their starch is not digested in the foregut and reaches the cecum. Consequently, these feeds can represent a threat to the balance of the hindgut ecosystem. Our results will help develop feeding programs to improve feed use and health care of horses.

Literature Cited


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