Mobile bag starch prececal disappearance and postprandial glycemic response of four forms of barley in horses

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ABSTRACT: To determine prececal starch digestibility and estimate glucose uptake from the digestion of 4 forms of barley in the small intestine, 4 mature cecally fistulated geldings (449 ± 41 kg BW) fed a 62:38 (wt/wt) meadow hay:concentrate diet at 1.7 kg DM/100 kg BW were included in a 4 × 4 Latin square design experiment. During each period, horses received 80% DM of their concentrate as 1 of the 4 forms of a same batch of barley, whole grain, 2.5 mm ground, steam flaked, and pelleted. Hay was offered in 2 equal meals and concentrate in 2 unequal meals. The starch supply in the morning meal amounted 2.7 g starch/kg BW. At each period, mobile bag DM and starch disappearance was determined. Except for ground barley, each form of barley was 4 mm ground before being introduced in the bag. Nylon bags containing each substrate were intubated in the horse receiving the pelleted barley. Bags were collected in the cecum for 10 h postintubation. At each period, postprandial glycemia was measured on blood samples collected on the 4 horses via an indwelling jugular catheter just before the concentrate morning meal and for 8 h. No hay in the morning meal was given the day of the measurements. Whole blood glucose was analyzed with a portable blood glucose meter. Mobile bag prececal DM disappearance and starch disappearance depended ($P < 0.01$) on barley form. Prececal starch disappearance of whole barley was the lowest but no difference ($P > 0.05$) was detected among the 3 processed grains. No significant effect of barley form was found whatever the glycemic parameters. No significant correlation was reported between glycemic parameters and the amount of prececal mobile bag disappeared starch calculated as the starch intake in the morning meal by the mobile bag starch disappearance. To conclude, the whole form of barley exhibited the lowest prececal mobile bag starch disappearance whereas, in relationship with large individual variations, no significant variation has been shown in glycemic parameters. Further investigations should be performed to improve methods for estimating prececal starch digestion of processed cereals in the different digestive segments of horses.

Key words: barley processing, glycemic response, horse, mobile nylon bag, prececal digestibility, starch

INTRODUCTION

Cereals are a common energy dietary source for exercising horses (Zeyner, 2008). The extent to which starch is digested in the prececal digestive tract not only determines the energy supply for the horse but may also affect its health status (Geor, 2010). This is why it is important to better determine prececal starch digestibility in horses.

In vivo measurements are scarce and showed limitations (Kienzle, 1994). Mobile bag technique on cecally fistulated equids has been proposed as an alternative (de Fombelle et al., 2004). A noninvasive method on nonfistulated horses, that is, the determination of glycemic and insulineic responses to a starchy meal, has also been used in numerous studies (Hoekstra et al., 1999; Richards et al., 2004; Vervuert et al., 2003, 2007) based on the assumption developed in humans that the rate of starch digestion and absorption of glucose in the small intestine determines the glycemic indices of cereals (O’Dea et al., 1981; Englyst et al., 1999). However, questions relative to the accuracy of glycemic response measurements for estimating starch digestibility in the small intestine remain important in humans (Eelderink et al., 2012).
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In horses, several studies were conducted to determine the impact of cereal source and process on glycemic response, but no relationships with prececal starch digestibility has been reported thus far (Vervuert and Coenen, 2006). On the other hand, mobile bag disappearance may vary according to starch botanic origin (de Fombelle et al., 2004; Rosenfeld and Austbo, 2009; Hymoller et al., 2012). But data concerning the impact of technological processing on prececal starch disappearance are scarce and controversial (Julliand et al., 2006; Rosenfeld and Austbo, 2009; Hymoller et al., 2012). But data concerning the impact of technological processing on prececal starch disappearance are scarce and controversial (Julliand et al., 2006; Rosenfeld and Austbo, 2009; Hymoller et al., 2012). The following experimental procedures were approved by the Ethics Committee for Animal Well-Being at the University of Burgundy, Dijon, France (agreement number A1309).

**MATERIALS AND METHODS**

The following experimental procedures were approved by the Ethics Committee for Animal Well-Being at the University of Burgundy, Dijon, France (agreement number A1309).

**Animals, Management, and Diets**

Four crossbred mature geldings, each fitted with a polyvinyl chloride cannula in the cecum and in the right ventral colon, were involved in a 4 × 4 Latin square design experiment. Horse’s mean age and BW were 11.5 ± 3.2 yr and 449 ± 41 kg, respectively. They were maintained in indoor individual free stalls bedded with wood shavings. They were dewormed (Eraquell-Virbac SA, Glattbrugg, Switzerland) 25 d before the beginning of the experiment. During the adaptation periods (17 d for the first period and 7 d for the next 3 periods), they had free access to sand paddock for 1 h/d 5 times a week and went to an automatic walker for 1.5 h/d 5 times a week. During the experimental periods (11 d), horses were turned out in a round dry pen 3 times per week for 30 min where they were led at a steady trot followed by a walk.

Horses were fed a meadow hay:concentrate (62:38; DM basis) diet at 17 g DM/kg BW. The concentrate contained an experimental pelleted feed rich in fiber (20% DM basis) and barley (80% DM basis) given as 4 different forms: whole grain, 2.5 mm ground, pelleted, and steam flaked, all of which came from the same batch of cereal. For the pelleted barley form, the ingredients of the experimental pelleted concentrate feed rich in fiber (20% DM basis). The chemical composition of feeds and the ingredients and chemical composition of experimental diets are presented in Tables 1 and 2, respectively. Horses had free access to water and a salt block.

**Table 1. Chemical composition of feed samples used in the study**

<table>
<thead>
<tr>
<th>Item</th>
<th>WB</th>
<th>GB</th>
<th>SF</th>
<th>PB</th>
<th>HF feed</th>
<th>Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>89.0</td>
<td>89.0</td>
<td>90.0</td>
<td>89.5</td>
<td>91.0</td>
<td>89.5</td>
</tr>
<tr>
<td>Starch, % DM</td>
<td>57.9</td>
<td>60.9</td>
<td>62.1</td>
<td>49.0</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>NDF, % DM</td>
<td>19.5</td>
<td>16.7</td>
<td>17.6</td>
<td>21.5</td>
<td>38.7</td>
<td>67.6</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>5.6</td>
<td>4.4</td>
<td>4.8</td>
<td>10.5</td>
<td>28.5</td>
<td>36.8</td>
</tr>
<tr>
<td>ADL, % DM</td>
<td>1.3</td>
<td>1.4</td>
<td>1.1</td>
<td>2.7</td>
<td>8.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

1WB = whole barley; GB = ground barley; SF = steam-flaked barley; PB = pelleted barley. All came from the same batch of barley.

2HF = High fiber. Experimental pelleted feed rich in fiber.

3Pelleted barley form was composed of barley (80% DM basis) and of the ingredients of the experimental pelleted concentrate feed rich in fiber (20% DM basis).

**Table 2. Ingredients and chemical composition of the diets (DM basis)**

<table>
<thead>
<tr>
<th>Item</th>
<th>W</th>
<th>G</th>
<th>SF</th>
<th>P</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients, % DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay</td>
<td>62.0</td>
<td>62.0</td>
<td>62.0</td>
<td>62.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole barley</td>
<td>30.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground barley</td>
<td>–</td>
<td>30.4</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steam-flaked barley</td>
<td>–</td>
<td>–</td>
<td>30.4</td>
<td>–</td>
<td>0.11</td>
<td>0.147</td>
</tr>
<tr>
<td>Pelleted barley²</td>
<td>–</td>
<td>–</td>
<td>38.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF feed³</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1W = diet based on whole barley concentrate; G = diet based on ground barley concentrate; SF = diet based on steam-flaked barley concentrate; P = diet based on pelleted barley concentrate; the 4 barley forms came from the same batch of cereal.

2Pelleted barley form was composed of barley (80% DM basis) and of the ingredients of the experimental pelleted concentrate feed rich in fiber (20% DM basis).

3HF = High fiber. Experimental pelleted feed rich in fiber.

x-yzWithin a row, means without common superscript letters differ, P < 0.05.

**Mobile Bags Measurements**

During each experimental period (on d 5), prececal starch digestibility of the 4 forms of barley was estimated using the mobile bag technique on the horse fed pelleted barley concentrate according to the procedure of de Fombelle et al. (2004). Mobile bags were prepared from polyester tissue (Laboratoire Humeau, La Chapelle sur Erdre, France; 50 μm porosity) and filled with 400 mg DM of each barley form. Except for ground barley, each substrate was previously ground to pass a 4-mm mesh before being introduced in the bag. Bags were intubated immediately after the ingestion of the third of the pelleted morning meal and they were washed out in...
the esophagus through a nasogastric tube with 500 mL of water (de Fombelle et al., 2004). The remainder of the meal was fed just after the intubation. The bags were then captured at their arrival in the cecum using magnets withdrawn through the cannula every hour until the first bags’ recovery and then every 0.5 h until 10 h after the intubation. Collected bags were rinsed rapidly under water and then stored at −20°C. This procedure was repeated twice at 48-h intervals. For each horse and each day of intubation, 20 bags were introduced in the oesophagus, that is, 5 bags per substrate.

After thawing at room temperature, bags were washed 3 times (one 5-min and two 3-min cycles) with demineralized water in a domestic washing machine (Camper’s version 4; Bob Home Handels GmbH, St Marien, Austria). Then, the residues were dried in a forced-air oven at 40°C to constant weight. Each bag was weighed to calculate the DM disappearance. The residues from bags containing the same substrate were then pooled by the horse.

The fraction of barley DM that may escape the bags after intubation before being digested or during the rinsing procedure was determined according to an adaptation of the procedure proposed by Philippeau and Michalet-Doreau (1997). It consisted of immersing 1 bag filled with each substrate in 50 mL distilled water and agitating it in a 39°C water bath for 2 h. After removal, bags were rapidly rinsed with distilled water and lost particles were recovered by filtration on a dried filter paper (8 μm). Then, the bag residues and the filters were dried in a forced air oven at 40°C to a constant weight. For each substrate, 5 bags underwent the complete procedure.

Postprandial Glycemic Response Test

During each period (on d 10), postprandial glyceria was assessed following the feeding schedule developed by Ralston (1992) and Vervuert et al. (2007), that is, only concentrate was fed in the morning meal. Glucose concentration was measured in whole blood using a single drop collected via an indwelling jugular catheter at baseline (before concentrate meal) and thereafter at 30-min intervals for 4 h after feeding and then at 60-min intervals for the next 4 h. Whole blood glucose was immediately determined with a portable blood glucose meter (One Touch Ultra 2; LifeScan, Issy Les Moulineaux, France).

Biochemical Analyses

Dry matter content was determined in hay, experimental feed rich in fiber, and barley forms by oven drying at 105°C to a constant weight. Cell wall content was estimated by the NDF method of Van Soest and Wine (1967) as modified by Association Française de Nor-

malisation (AFNOR, 1997). Lignocellulose (ADF) and lignin (ADL) were obtained using a sequential approach (AFNOR, 1997) on the NDF. Barley forms, experimental feed rich in fiber, and in sacco residues were analyzed for starch content by a standard method (AFNOR, 2005). Gelatinization of starch was determined by Lareal Lab (In Vivo NSA, Saint Nolff, France).

Calculations and Statistical Analyses

For each period, the proportion of bags collected and the retention time of bags in the prececal digestive tract were calculated according to de Fombelle et al. (2004). Mean blood glucose concentration, time to peak, and peak value were calculated according to Vervuert et al. (2009b). Incremental area under the blood glucose curve (AUC) was determined using simple nonoverlapping polygons. Only the area over the baseline without the area beneath the curve was considered.

The variance homogeneity of DM disappearance, starch disappearance, and glycemic parameters was assessed using the Shapiro-Wilk test in Minitab (Minitab Inc., State College, PA). An ANOVA was conducted using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). Chemical compositions of diets were analyzed in a model including period and barley form as fixed effect and horse, as random effect. To assess the effect of barley form on prececal DM and starch disappearance, the model included barley form as fixed effect and horse as random effect. For glycemic variables, the model included period and barley form as fixed effects and horse as random effect. For DM losses, the model included barley form. Least squares means were calculated for each variable and separated using pairwise t tests when significant effects due to barley form were found (PDIFF option of SAS).

Pearson correlation coefficients were calculated for the comparisons between the intake of prececal digestible starch (i.e., starch intake in the morning meal × mobile bag starch disappearance) and glycemic variables.

Significance was declared at $P < 0.05$ and a trend toward significance at $P ≤ 0.10$.

RESULTS

Starch intake in the morning meal averaged 2.7 g/kg BW. Gelatinization of starch was the greatest for steam-flaked (33%) and pelleted (28%) barley whereas the lowest values were found for whole (19%) and ground (18%) barley.

Mobile Bag Dry Matter and Starch Disappearance

On average, 83.3 ± 2.9% of the intubated bags were recovered in the cecum during the 10-h collecting pe-
Period. Prececal retention time averaged 6.6 h and did not differ significantly \((P = 0.667)\) depending on the barley form (Fig. 1).

Dry matter disappearance depended \((P < 0.01)\) on barley form (Table 3). It was the lowest \((P < 0.01)\) for whole barley but did not differ significantly between steam-flaked and ground \((P = 0.239)\) or pelleted \((P = 0.192)\) barley, respectively. Dry matter disappearance of ground barley was greater \((P = 0.025)\) than of pelleted barley.

Starch disappearance depended \((P < 0.01)\) also on barley form. It was lower \((P < 0.01)\) in whole form than in processed grain, but no significant difference in starch disappearance among the 3 processed forms was determined \((P > 0.10)\) when barley processed forms were compared 2 by 2.

Dry matter losses through the pores of bags, independently of any digestive process, were determined in a water bath (Fig. 2). Total DM losses were \((P < 0.01)\) the greatest in pelleted barley. However, in processed barleys, no significant \((P = 0.128)\) effect of barley form was detected in the extent of DM losses recovered by filtration.

**Postprandial Glycemic Parameters and Relationship with Mobile Bag Starch Disappearance**

Regardless of the postprandial glycemic parameters, no significant effect \((P > 0.05)\) of barley form was found (Table 4). Whatever the glucose variable, no \((P > 0.05)\) correlation was detected with prececal digestible starch intake in the morning meal (Table 5). For the same value of prececal digestible starch intake in the morning meal, a great variability of the AUC was observed according to individual response (Fig. 3).

**DISCUSSION**

The present study aimed at measuring simultaneously the mobile bag disappearance and the postprandial glucose response in horses for 4 different forms of barley grains. Regardless of the method and the animal species, assessing the impact of physical processing on starch digestion based on literature data is difficult due to differences in botanical origin and conditions of the grain processing (temperature, duration, and/or pressure), which vary according to the authors (Svihus et al., 2005). In our study, to relate variation in prececal starch digestibility only to barley treatments, the 4 forms were prepared from the same cereal batch.

A concordance was shown between the 2 methods we used for estimating whole barley prececal digestion. Indeed, whole barley exhibited the lowest DM disappearance, the lowest starch disappearance, and, numerically, the lowest AUC. Due to the physical barrier made of pericarp and protein matrix surrounding starch granules and little damage in starch granules structure, the accessibility of starch granules to amylase was limited (Kienzle, 1994). Therefore, whole grain had a lower prececal starch digestibility than processed forms and may lead to a numerical lower entry of glucose in the bloodstream. Similar to what is done for other monogastric animals, physical processing of cereal grains is used in horses to increase prececal starch digestion of grain and they may contribute to limit the arrival of nondigested starch in the hindgut that can potentially disturb the microbial ecosystem (Julliand et al., 2001, 2006). Grinding in-

**Table 3. Mobile bag DM disappearance and starch disappearance of 4 barley forms in the prececal part of the digestive tract in horses fed pelleted barley grain**

<table>
<thead>
<tr>
<th>Item</th>
<th>WB</th>
<th>GB</th>
<th>SFB</th>
<th>PB</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>44.7(^x)</td>
<td>74.6(^y)</td>
<td>72.3(^z)</td>
<td>69.6(^z)</td>
<td>1.87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Starch, %</td>
<td>55.1(^x)</td>
<td>97.4(^y)</td>
<td>94.1(^y)</td>
<td>92.8(^y)</td>
<td>3.87</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(^{x-\text{z}}\)Within a row, means without common superscript letters differ, \(P < 0.05\).

1\(\text{WB} = \text{whole barley}; \text{GB} = \text{ground barley}; \text{SFB} = \text{steam-flaked barley}; \text{PB} = \text{pelleted barley}.\) All came from the same batch of barley.
creased starch disappearance in comparison with whole grain due to particle size reduction. Thermomechanical processing methods such as pelleting and steam flaking are expected to provide the most alterations of the native grains and to increase starch digestion. Steam flaking uses moisture and pressure to disrupt the protein matrix around starch granules and hydrogen bonds in starch granules (Svihus et al., 2005), which is in accordance with the greatest starch gelatinization for steam-flaked barley we measured. For pelleted barley, starch gelatinization consecutive to shear forces occurring during pelleting is less marked. For these 2 thermomechanical treatments, disruption of hydrogen bonds and water absorption in gelatinized starch fraction may improve enzymatic digestion of starch granules (Svihus et al., 2005). However, we did not detect any difference in mobile bag starch disappearance and AUC between ground grains and thermomechanical processed forms. This result concurred with the mobile bag results reported by Rosenfeld and Austbo (2009), who found no variation in starch disappearance between 1 mm ground and pelleted forms whatever the cereal (oat, barley, and corn) whereas Hy-moller et al. (2012) showed a greater prececal starch disappearance for flaked than coarsely ground corn, that is, cracked form. Concerning the glycemic response, discordant results were reported in the literature attributable to difference in the particle size of ground cereals or the level of starch intake (Vervuert and Coenen, 2006). For a starch intake of 2 g starch/kg BW per meal, the ground form of corn led to lower area under the glucose curve and peak value than the steam-flaked form (Hoekstra et al., 1999) and cracked barley showed a lower glycemic response than pelleted form (Nielsen et al., 2010). For a lower starch intake varying between 1.2 to 1.5 g/kg BW, no difference (P < 0.05) was detected between finely ground (2-mm sieve) and steam-flaked barley (Vervuert et al., 2007). Regarding the 2 thermomechanical treatments we tested, no significant difference in mobile bag starch disappearance between steam-flaked and pelleted forms was reported whereas pelleted barley exhibited numerically the greatest AUC. In horses, prececal starch disappearance may result both from starch fermentation in the stomach in lactic acid and enzymatic hydrolysis in the small intestine in glucose (Varloud et al., 2007). Based on results of O’Dea et al. (1981), the lower numerical glycemic response assessed in controlled conditions for steam-flaked form may reflect the lower rate at which starch is hydrolyzed in glucose and absorbed in the small intestine. Therefore, nonnegligible starch fermentation may occur in the stomach for steam-flaked barley in comparison with pelleted barley. This hypothesis is reinforced by 2 literature data. Hoekstra et al. (1999) showed a peak of lactic acid in blood occurring rapidly after a steam-flaked corn intake and the area under the blood lactate curve was greater for this form than ground corn. Al Jassim (2006) also confirmed that a greater lactic acid concentration was measured in the nonglandular stomach of horses fed steam-flaked rather than dry-rolled sorghum. The increase in the volume intake of steam-flaked form might have been associated with a slower gastric emptying in horses (Métayer et al., 2004). Consequently, difference in retention time in the stomach could affect the time of exposure of starch to

![Figure 3](image-url)
the bacteria enzymes and the extent of starch fermentation in this digestive segment. More data are necessary to confirm this hypothesis, which may impact common assumption; that is, a low glycemic response could reflect a great amount of starch escaping to the hindgut (Nielsen et al., 2010).

Interestingly, for a same value of prececal digestible starch intake in the morning meal, we observed a great numerical variability of the AUC (Fig. 3). This can be explained by differences in the partition of starch digestion in the prececal digestive tract as we discussed previously. However, these results should be considered with caution because no standardized procedures have been established yet for these 2 alternative methods. To increase their statistical power, further methodological investigations have to be performed. Regarding postprandial glycemic response, we found large individual variations in the area under the glucose curve for a similar barley form as it was already reported by Vervuert et al. (2008, 2009a, b). To bring more explanations to glycemic response, insulinenic response should be measured. According to Nielsen et al. (2010), both glycemic and insulinenic responses are altered with cereal processes. In the present study, we did not suspect difference in insulinenia resistance of tested horses during the experiment because variations coefficients of individual mean BW were negligible. For further investigations, postprandial concentrations of insulin and blood energetic end products such as organic acids and glucose could be assessed in peripheral venous simultaneously to quantify starch digestion in the different segments of the digestive tract in equids.

Regarding the mobile bag technique, weaknesses could be also reported. In this technique, the assumption is that the DM fraction remaining in the bag corresponds to the undigested DM fraction and, as a consequence, all particles leaving the bag are digested. However, to mimic reduction size of particles during mastication and to obtain a homogenous sample, substrates are ground before being introduced in the nylon bags (Nozière and Michalet-Doreau, 2000). This previous grinding may lead to a bias in the determination of prececal starch disappearance according to the physical treatment. In the present study, whereas 2.5 mm ground barley was not submitted to further grinding, whole, steam-flaked, and pelleted barley grains were ground through 4-mm sieve before their introduction in the bags. This preliminary treatment probably increased the fine particle DM fraction (<50 μm) that then escaped from the bags as mechanical losses. Few in sacco studies in horses focused on DM losses through the bags’ pores. In the present study, the lost fraction, which we determined using a water bath, could be divided into soluble and particulate losses with a diameter higher than 8 mm (Nozière and Michalet-Doreau, 2000). The soluble fraction (total minus particles recovered by filtration) represents the rapidly digested starch fraction in the prececal digestive tract, which can be potentially fermented by the bacteria in the stomach before being enzymatically digested in the small intestine. For this soluble fraction, we hypothesized that the digestion rate occurring in the digestive tract was similar to the outflow rate from the bags. As a consequence, the variation in this soluble fraction should not affect the extent of starch disappearance. On the contrary, DM fraction recovered by filtration might escape from the nylon bag more rapidly than its potential digestion rate in the bag. Therefore, this fraction could potentially lead to an overestimation of starch disappearance. In the present study, this fraction remained low despite the barley form and most likely did not affect mobile bag disappearance. In ruminants, de Jonge and Dijkstra (2011) reported that DM loss through the bags’ pores estimated by an inert substrate, silica gel, was more important during the rinsing procedure than in the ruminal content. Further methodological studies have to be conducted in horses to test this hypothesis. In this respect, a washing-machine step could be added in the procedure of losses measurement to verify if additional losses could be observed.

In conclusion, processed barley forms exhibited greater mobile bag starch disappearance whereas, in relationship with large individual variations, no difference in glycemic parameters has been detected. Based on our results, these 2 methodologies did not allow us to classify processed barley forms. Further methodological investigations are required to increase knowledge concerning digestive mechanisms such as starch fermentation in the stomach, to define standardized procedures for estimating prececal starch digestibility, and to provide consensual recommendations regarding the upper level of starch intake per meal for preventing health disturbances and optimizing energy supply for horses.

**LITERATURE CITED**


Prececal starch digestion in horses


