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Impact of barley form on equine total tract fibre digestibility and colonic microbiota

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This study aimed at assessing the impact of four barley forms on total tract apparent digestibility of dietary fibre in horses fed a large amount of starch in the morning meal (0.27% BW). Processed barley forms had a greater pre-caecal starch digestibility than the whole form. Based on this result, we hypothesised that using barley-processing methods would limit the potential dumping of undegraded starch in the hindgut of horses and, consequently, the potential negative effect on fibre degradation in the hindgut. In a 4 × 4 latin square design, four mature geldings fitted with a right ventral colon-fistula were fed a meadow hay : concentrate (62 : 38; dry matter (DM) basis) diet at 1.7% BW. The concentrate was made of 80% barley distributed either as whole grain or as processed forms: 2.5 mm ground, pelleted or steam-flaked. For each period, total tract apparent digestibilities of DM, NDF and ADF were determined over 3 consecutive days by total faecal collection, whereas pH, volatile fatty acids (VFA) concentrations and cultural functional bacteria counts (total anaerobic, cellulolytic bacteria, lactic acid producers, amylolytic bacteria and lactic acid utilisers) in colonic content were evaluated on 1 day 4 h after the morning meal. Total tract apparent digestibility of DM and dietary fibre was influenced (P < 0.05) by barley form. Diets including thermo-mechanically treated barley forms led to a higher (P < 0.05) total tract apparent digestibility of NDF than those constituted of ground barley and also led to a greater (P < 0.05) total tract apparent digestibility of ADF than those made of whole or ground barley forms. However, no significant difference was observed in colonic pH, VFA concentrations and cultural bacteria concentrations. Owing to a high starch supply in the morning meal, the concentration of the functional bacteria in the colonic content averaged 7.8 log CFU/ml, 5.9 NPM/ml, 6.9 and 7.3 CFU/ml for total anaerobic, cellulolytic, amylolytic and lactic acid-utilising bacteria, respectively. Consequently, providing horses with pelleted or steam-flaked instead of ground barley forms may limit the negative impact of starch on fibre digestibility in horses fed a high level of starch in the morning meal (0.27% BW). Moreover, the fibre-to-starch ratio fed in this experiment did not cause any digestive upset.

Keywords: horse, digestion, starch, fibre, hindgut

Implications

Cereals are a common energy dietary source for exercising horses. Based on these results, using thermo-mechanically processed barley instead of ground barley may lead to a higher dietary fibre digestion. Consequently, feeding horses with thermo-mechanically processed barley may constitute an interesting feeding strategy for exercised horses receiving diets rich in starch.

Introduction

In horses, starch is a major energetic component of the diet as its digestion in the small intestine provides glucose. However, the enzymatic capacity of the small intestine to

hydrolyse starch is limited in horses because of low amylase secretion in comparison with other animal species (Alexander and Hickson, 1970; Kienzle, 1994). In this respect, feeding at least 0.35% (Potter *et al.*, 1992), 0.3% (Zeyner, 2008) or even 0.2% (Julliand *et al.*, 2006) BW per meal may lead to a dumping of undigested starch in the hindgut. In this digestive segment, the rapid microbial fermentation of starch produces organic acids, which can lead to a decrease in pH and depress the proportion of cellulolytic bacteria and the cellulolytic activity (Julliand *et al.*, 2001). At times, the energetic supply from forage degradation may decrease and, ultimately, microbial disturbances may occur in the hindgut and be responsible for digestive upsets (Daly *et al.*, 2012) such as colic (Hudson *et al.*, 2001; Archer and Proudman, 2006).

The range in the recommendation for the upper level of starch per meal reported in the literature probably results from variation in the extent of pre-caecal starch digestibility,

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which largely depends on starch sources (De Fombelle *et al.*, 2004; Rosenfeld and Austbo, 2009; Hymoller *et al.*, 2012) and physicochemical forms of cereal grains (Rosenfeld and Austbo, 2009; Hymoller *et al.*, 2012). Using the same batch of barley, a greater mobile bag pre-caecal starch disappearance was found for ground, pelleted or steam-flaked forms than for whole grain (Philippeau *et al.*, 2014). Providing processed barley forms to horses could prevent the depression of cellulolytic bacteria in mixed diet, thus enhancing digestibility of dietary fibre in the total digestive tract and limiting the occurrence of microbial ecosystem disturbances in the hindgut. However, these hypotheses have been controversial in the bibliography for diets based on processed forms of barley and sorghum (McLean *et al.*, 1999; Al Jassim, 2006). The aim of the present study was to determine the impact of the four barley forms we previously studied (Philippeau *et al.*, 2014) on fibre total tract apparent digestibility and on the fibrolytic activity and bacteria in the colon of horses.

Material and methods

Experimental design

The experiment was designed as a 4 × 4 Latin square with four horses and four experimental periods. Each period consisted of 7 days of adaptation to the diet and 11 days of data collection. For the first experimental period, the duration of the adaptation period was longer (17 days) than the three others to allow a gradual increase in starch proportion in the horse ration. The Ethics Committee for Animal Well-Being at the Faculty of Burgundy, Dijon, approved the following experimental procedures (A1309).

Horses

Four cross-bred mature geldings (11.5 ± 3.2 years old; 449 ± 41 kg) each fitted with a polyvinyl chloride cannula in the caecum and in the right ventral colon were included for the study. They were maintained indoors in individual free stalls bedded with wood shavings. They were dewormed

(Eraquell®; Virbac SA, Glattbrugg, Switzerland) 25 days before the beginning of the experiment. During the adaptation periods to the diet, they had access to a sand paddock for free exercise for 1 h/day five times per week, and they used an automatic walker for 1.5 h/day five times per week. During the periods of data collection, horses were taken out to a round dry pen three times per week for 30 min, where they walked for 5 min, followed by a steady trot for 20 min and walked again for 5 min.

Feeding management

The diets were formulated to meet the recommendations for horses subjected to 1 h very light work as defined by Institut National de la Recherche Agronomique (Martin-Rosset, 1990) and to supply >1 kg dry matter (DM) hay per 100 kg BW. Horses were fed a meadow hay : concentrate (62 : 38; DM basis) diet at 1.7 kg DM/100 kg BW. The concentrate was composed of an experimental fibrous pelleted feed (20% DM basis) and barley (80% DM basis). Barley came from a single batch of cereal and was fed either as whole grain, 2.5-mm ground, steam-flaked or pelleted (Table 1). Hay was provided in two equal meals at 1000 and 1700 h. The concentrate was fed in two unequal meals (5/6 and 1/6) at 0800 and 1730 h, allowing a starch intake in the morning meal higher than 0.2% BW (Julliand *et al.*, 2006). Salt blocks and water were available *ad libitum*.

To characterise starch sources and to estimate the potential starch dumping in the hindgut, starch gelatinisation and pre-caecal starch digestibility estimated by starch mobile bag disappearance between the oesophagus and the caecum had been measured on these four barley forms in this experiment (Philippeau *et al.*, 2014) (Table 1).

Total tract apparent digestibility measurements

Each data collection period began with the measurement of *in vivo* total tract apparent digestibility using a 3-day total collection of faeces (Goachet *et al.*, 2009). Faeces samples were collected using a collection harness to which horses had been previously adapted to wearing. They were emptied three times a day. For each horse, the amount of feed

Table 1 Chemical composition (% of the DM) of the feed samples and starch characteristics of the four barley forms used in the study

Item	Barley form ¹				Fibrous feed ³	Hay
	Whole	Ground	Steam-flaked	Pelleted ²		
Starch	57.9	60.9	62.1	49.0	5.0	0.0
NDF	19.5	16.7	17.6	21.5	38.7	67.6
ADF	5.6	4.4	4.8	10.5	28.5	36.8
ADL	1.3	1.4	1.1	2.7	8.7	3.4
Starch D (% starch) ⁴	55.1	97.4	94.1	92.8	–	–
Gelatinisation (% starch) ⁵	19	18	33	28	–	–

DM = dry matter.

¹All 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 feed rich in fibre (20% DM basis).

³Experimental pelleted feed rich in fibre.

⁴Mobile bag pre-caecal starch disappearance (Philippeau *et al.*, 2014).

⁵Starch gelatinisation (Philippeau *et al.*, 2014).

offered, refused and faeces were weighed every day. Aliquots of individual faeces samples (10% of total excreted fresh faeces), offered feeds (500 g of fresh matter) and all refusals, if any, were taken daily, dried and ground. At the end of each period, representative samples from each collection of offered feeds, refusals and faeces were pooled for each horse over the 3-day period for subsequent analysis.

Faecal, feed refusals and feed samples were dried in a forced-air oven at 60°C until constant weight was achieved for DM determination. NDF, ADF and ADL were determined according to Van Soest and Wine (1967) as modified by Association Française de Normalisation (AFNOR) (1997). Starch content was determined by an enzymatic method (AFNOR, 2005).

Colonic abiotic and biotic parameters determination

On day 9 of data collection, 4 h after the morning meal, colonic contents were collected using gravity via the cannula, and the pH was immediately measured using an electronic pH-meter (MP 120; Mettler, Toledo, Spain). Subsequently, aliquots of colonic contents were filtered through a 100-µm sieve nylon filter (Bluetex; SAATI, Saily-Saillisel, France) and frozen (-20°C) for further biochemical measurements. Total volatile fatty acid (VFA) concentrations were determined by GLC (Clarus 500 PPC; PerkinElmer, Courtaboeuf, France), following the procedure described by Jouany (1982). For microbial analyses, one aliquot of colonic contents was held in CO₂-saturated flasks at 38°C before inoculation. Microbial analyses were carried out as described by Julliard *et al.* (1999) and Faubladiet *et al.* (2013). Ten-fold dilution series were prepared under O₂-free CO₂ in an anaerobic mineral solution. Total viable anaerobic bacteria counts were determined using a modified non-selective medium in anaerobic roll tubes. Lactic acid-utilising bacteria counts were determined in a selective medium in anaerobic roll flasks. For these two flora, the number of viable bacteria was determined after 48 h of incubation at 38°C as the average of colony counts from three replicate roll tubes prepared from dilutions representing 10⁻⁵, 10⁻⁶ and 10⁻⁷ ml of diluted colonic effluent. Concentrations of cellulolytic bacteria were determined using a modified broth medium after 15 days of incubation at 38°C as the most probable number on four roll tubes inoculated with dilutions representing 10⁻⁵, 10⁻⁶ and 10⁻⁷ ml of colonic contents. Amylolytic bacteria were deeply inoculated on an adapted medium containing 1% (w/v) of soluble starch as the main energy source. Three replicate Petri plates were inoculated per dilution representing 10⁻⁴, 10⁻⁵ and 10⁻⁶ ml of diluted colonic effluent. For bacterial counts, iodine solution was added to the Petri plates after 48 h incubation at 38°C.

Calculations

Total tract apparent digestibility coefficient of DM was evaluated using ADL as the internal marker as follows:

$$\text{DMd} (\%) = \left(1 - \frac{A}{B}\right) \times 100$$

where DMd was the DM digestibility, *A* and *B* were the ADL concentrations in the intake and faeces, respectively.

Total tract apparent digestibilities of DM constituents were calculated as follows:

$$\text{Constituents digestibility} (\%) = \left[\text{DMd} \times \left(\frac{\text{CI}}{\text{CF}} \right) \right] \times 100$$

where CI and CF were the DM constituent concentrations in the intake and the faeces, respectively.

Microbial counts were expressed as log₁₀ of colony-forming units (CFU) per millilitre.

Statistical analysis

The variance homogeneity of variables was assessed using the Shapiro–Wilk test in Minitab (Minitab Inc., State College, PA, USA). All the variables were analysed using the MIXED procedure of SAS v9 (SAS Institute Inc., Cary, NC, USA). Daily nutrient intakes, digestibility coefficients, pH values, organic acid concentrations and microbial counts were analysed in a model, including period and barley form as fixed effects and horse as the random effect. Least square means were calculated for each variable and separated using pair-wise *t*-tests when significant effect due to barley form was found (PDIF option of SAS). Significance was declared at *P* ≤ 0.05 and a trend towards significance at *P* ≤ 0.10.

Results

DM and starch intakes averaged 1.7% and 0.32% BW, respectively. The average starch intake in the morning meal amounted to 0.27% BW.

Total tract apparent digestibility of DM depended (*P* = 0.043) on the barley form used in the concentrate (Table 2). It was greater for diets including pelleted than that for whole and ground barley forms and was intermediate for steam-flaked diet. Total tract apparent digestibility of starch was almost complete whatever the barley form and averaged 99%. For insoluble dietary cell wall fraction (NDF), total tract apparent digestibility was (*P* = 0.033) the lowest for the diet including ground barley and the highest for thermo-mechanical barley forms. However, no difference between the whole form and each of the other forms was detected.

Table 2 Effect of barley form on total tract apparent digestibility of DM constituents

Item (%)	Barley form				SEM	<i>P</i> -value
	Whole	Ground	Steam-flaked	Pelleted		
DM	71.8 ^a	70.9 ^a	73.9 ^{ab}	75.1 ^b	1.2	0.04
Starch	97.7	100.0	100.0	100.0	1.0	0.12
NDF	61.0 ^{ab}	58.4 ^a	62.9 ^b	62.7 ^b	1.2	0.03
ADF	52.0 ^a	49.8 ^a	54.0 ^b	54.0 ^b	1.2	0.03

DM = dry matter.

^{a,b}Least square means within the same row lacking a common superscript letter are different (*P* < 0.05).

Table 3 Effect of barley form on biotic and abiotic parameters of colonic content collected 4 h after the morning meal

Parameter	Barley form				SEM	P-value
	Whole	Ground	Steam-flaked	Pelleted		
Bacteria						
Anaerobic bacteria (log CFU/ml)	7.65	8.14	8.17	7.33	0.17	0.19
Cellulolytic bacteria (NPM/ml)	6.02	5.21	6.13	6.15	0.15	0.17
Amylolytic bacteria (log CFU/ml)	7.05	6.91	6.30	6.91	0.20	0.41
Lactic acid-utilising bacteria (log CFU/ml)	7.07	7.55	7.74	6.85	0.19	0.29
pH						
Value	6.32	5.91	6.25	6.08	0.08	0.36
VFA						
Total VFA (mmol/l)	108.3	91.7	102.1	87.3	5.6	0.50
Acetate (mmol/l)	75.6	62.0	72.4	63.1	4.3	0.52
Propionate (mmol/l)	24.2	22.5	22.1	18.3	1.0	0.32
Butyrate (mmol/l)	5.4	5.1	6.3	4.4	0.8	0.83
(Acetate + butyrate) : propionate	3.49	2.96	3.62	3.68	0.34	0.24

VFA = volatile fatty acid.

Total tract apparent digestibility of ADF was greater in diets including thermo-mechanical barley forms compared with whole and ground barley forms ($P = 0.030$).

No difference in pH ($P = 0.360$) and VFA concentrations ($P = 0.502$) was reported according to the barley form. The pH and total VFA concentrations averaged 6.1 and 97.4 mmol/l, respectively (Table 3). As it was reported for abiotic parameters, no difference was detected in total anaerobic bacteria concentration and its composition, using the cultural method (Table 3). The concentration averaged 7.8 log CFU/ml, 5.9 NPM/ml, 6.9 and 7.3 CFU/ml for total anaerobic, cellulolytic, amylolytic and lactic acid-utilising bacteria, respectively.

Discussion

In horses, interactions between starch and total tract apparent digestibility of dietary fibre have been studied more extensively in trials assessing variable proportions of starch in the DM intake. Controversial results have been obtained, which may be due to differences between experimental designs. Increasing levels of starchy concentrate in the diet of horses at the expense of the hay proportion led to a decrease in the total tract apparent digestibility of fibre fractions (Vermorel *et al.*, 1997; Palmgren-Karlsson *et al.*, 2000) or an increase (Vermorel *et al.*, 1997). As the proportion of concentrate remained constant, the increase of starch level in the concentrate at the expense of NDF level led to either a decrease (Jouany *et al.*, 2008) or an increase in fibre total tract apparent digestibility (Swyers *et al.*, 2008). For increasing DM intake, no impact on NDF total tract apparent digestibility was found between a hay-based diet and a hay : barley diet (Hussein *et al.*, 2004). In the present study, interactions between starch and degradation of dietary fibre in the hindgut were studied for different processed barley forms. Horses fed thermo-mechanical barley forms exhibited

greater digestibility of ADF compared with horses receiving whole grain or mechanically treated barley forms. This result may suggest that diets including thermo-mechanical cereal forms may lead both to a higher starch digestibility in the pre-caecal digestive tract and a higher fermentation of dietary fibre in the hindgut. Few results are available in the literature regarding the impact of cereal processing on fibre digestibility and controversial data are reported (McLean *et al.*, 1999; Al Jassim, 2006). In one study, the physical form (rolled, micronised and extruded) of barley-based diets did not affect NDF and ADF total tract apparent digestibility (McLean *et al.*, 1999). In another study, a lower total tract apparent digestibility of ADF was found for steam-flaked than for rolled forms of sorghum-based diets (Al Jassim, 2006). Based on these two studies, it appears difficult to classify cereal processed forms according to their potential deleterious impact on fibre digestibility.

Al Jassim (2006) reported significant lower total VFA concentration and higher proportion of propionate in faeces of horses, showing the lower total tract digestibility of dietary fibre. However, no clear relationship was established between total tract apparent digestibility and abiotic parameter concentrations in the hindgut content measured at 4 and 5 h after feeding by McLean *et al.* (1999 and 2000). In the present study, the differences in total tract apparent digestibility of dietary fibre were consistent with numerical variations in pH, VFA concentrations and bacteria counts in colonic content. Thus, horses receiving ground barley showed the lowest *in vivo* digestibility of dietary fibre and numerically lower values in pH, cellulolytic bacteria count and ((C2 + C4)/C3) ratio.

In comparison with other studies dealing with the impact of cereal processing on horse digestibility, the pre-caecal starch digestibility of the four barley forms we tested had been previously determined (Philippeau *et al.*, 2014), using the mobile bag technique. In that study, the thermo-mechanical barley forms exhibited a greater pre-caecal

starch digestibility compared with whole grain. Based on these *in sacco* results, starch in whole barley was not entirely digested in the pre-caecal tract and, consequently, may have been flushed in the hindgut. In the caecum, starch fermentation may be limited because fine particles flow quickly through the caecum (de Fombelle *et al.*, 2001). However, starch may remain longer in the colon where it may be fermented and may interact with degradation of dietary fibre. This hypothesis is in agreement with the data of the present study. Surprisingly, although mobile bag pre-caecal starch disappearance of ground barley was higher compared with whole grain (Philippeau *et al.*, 2014), no statistical difference in total tract apparent digestibility of NDF and ADF was detected between these two treatments. For a starch intake in the morning meal higher than 0.2% BW, we would have expected that the large variation in the extent of starch disappearance (almost 40 points) may lead to a significant difference in the amount of starch that entered the hindgut and may affect fibre digestibility as it was suggested by Julliand *et al.* (2006). Although conclusions based on the nylon bag technique data should be studied with caution, undigested particulate DM losses through nylon bag pores remained low whatever the barley form (Philippeau *et al.*, 2014) and could not insert bias in the mobile bag technique. Consequently, according to us, the discrepancy between *in sacco* and *in vivo* results may be related to difference in particle size reduction of barley forms. In contrast to the *in sacco* technique, barley forms were subjected, *in vivo*, to chewing activity. Possibly, due to the differential chewing activity according to the physical forms of cereal, mean particle size of the digesta did not differ, and, consequently, *in vivo* starch accessibility of whole and ground barley forms to microorganisms' enzymes in the stomach and pancreatic amylase may be similar. If faecal particle length and width were used as the indicator of chewing efficiency, our hypothesis could be corroborated by data from the study by Brokner *et al.* (2008), who showed no difference between horses receiving whole and ground oats as concentrates.

No data dealing with the impact of cereal form on functional bacteria concentrations are reported in the literature. Irrespective of the barley form (whole *v.* processed forms), culture-based bacterial measurements in colonic content showed high concentrations of amylolytic and lactic acid-utilising bacteria. This result is in accordance with values for streptococci, lactobacilli and lactic acid-utilizing bacteria reported in horses receiving a high-starch diet (Julliand *et al.*, 2001; Medina *et al.*, 2002; de Fombelle *et al.*, 2003). Surprisingly, owing to acidic conditions of colonic contents, cellulolytic bacteria counts remained, whatever the barley form, higher than values reported previously in horses receiving a high-starch diet (Julliand *et al.*, 2001; Medina *et al.*, 2002; de Fombelle *et al.*, 2003). At pH values ranging from 5.9 to 6.3, degradation of fibre may be altered in ruminal content (Erdman, 1988), resulting from an inappropriate bacterial adhesion to the dietary fibre and/or a slower growth of cellulolytic bacteria (Weimer, 1996). Because the deleterious impact of acidic pH on fibre digestion depends on duration

and intensity of the pH decrease, further investigations based on repeated measurements should be proposed to assess the effect of barley form on post-prandial pH dynamics in colonic content of horses. In a similar concentrate feeding strategy and proportion of concentrate in the diet, we showed that the pH tended to decrease 4 h after feeding, but it was the lowest from 6 to 7 h post-feeding (Philippeau *et al.*, 2009). Moreover, the links between pH and main cellulolytic microorganisms in horses, such as fungi, protozoa and the major bacteria strains *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*, should be investigated using molecular techniques. To explain the high cellulolytic bacteria concentration, irrespective of the barley form, other diet characteristics should be considered in the present study. The high starch inclusion in the morning meal may have been counteracted by the low starch inclusion in the evening meal. Consequently, the starch concentration remained low (in average, 17% of DM intake). On the other hand, the forage DM intake was higher (1.2% BW) than that in previous studies reporting deleterious impact of starch on cellulolytic bacteria (Julliand *et al.*, 2001; de Fombelle *et al.*, 2003). Sufficient intake of physical effective fibre may constitute an abundant substrate for cellulolytic microorganisms in the hindgut and stimulate their growth.

In conclusion, using thermo-mechanical cereals in diets rich in starch may limit potential negative impact of starch on fibrolytic activity in horses. Moreover, the fibre-to-starch ratio fed in this trial did not cause any digestive upset, although morning meal starch intake was higher than 0.2% BW. Further studies should be carried out to determine the optimum value of the dietary fibre-to-starch ratio for athletic horses that may limit the risk of hindgut disorders without impairing their exercise performances.

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