NON RUMINANT NUTRITION

The effects of processing barley and maize on metabolic and digestive responses in horses

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Abstract

The competition for customers increases the search for new grain processing methods for equine feed, but the effect on starch digestibility and metabolic responses varies. Therefore, to evaluate the effect of the processing methods, toasting and micronizing, on starch digestion and the effect on metabolic responses, the mobile bag technique (MBT) and plasma glucose and insulin concentrations in the blood were used to estimate nutrient disappearance and metabolic responses pre-cecally. Further, cecal pH, ammonium nitrogen (N), and short-chain fatty acid (SCFA) concentrations were used to estimate the metabolic response in the cecum. Four cecally cannulated horses (body weight [BW] 565 ± 35 kg) were used in a 4 × 4 Latin square design with four periods of 8 d of diet adaptation and 2 d of data collection. Diets were formulated using hay and processed grains: micronized barley (MB), toasted barley (TB), micronized maize (MM), and toasted maize (TM) and were balanced to provide 1 g starch/kg BW in the morning meal. On day 9 in each period, blood and cecal fluid samples were taken before the morning meal and hourly thereafter for 8 h. On day 10 in each period, 15 bags of either MB, TB, MM, or TM (1 × 1 × 12 cm; 15 µm pore size; 1 g feed) were placed in the stomach, respectively. The dry matter disappearance was highest for the MM at all time points compared with the other feedstuffs ($P < 0.001$). Maize and micronizing had the highest starch disappearance ($P = 0.048$) compared with barley and toasting. No treatment effect was measured for any of the glucose and insulin parameters. No feed effect was measured for the insulin parameters. Plasma glucose peaked later ($P = 0.045$) for maize than for barley, and TB had a larger area under the curve for glucose than MB, MM, and TM ($P = 0.015$). The concentration of total SCFA increased after feeding ($P < 0.001$), with a higher concentration for barley than for maize ($P = 0.044$). No treatment or feed effects were measured for ammonium N or pH, but both were affected by time ($P < 0.001$). In conclusion, toasting was not as efficient as micronizing to improve pre-ecal starch digestibility; therefore, the preferred processing method for both barley and maize is micronizing. Further, the amount of starch escaping enzymatical digestion in the small intestine was higher than expected.

Key words: glucose, insulin, mobile bag technique, pH, short chain fatty acid

Introduction

The apparent total tract digestibility of starch in grains is found to be nearly 100% in horses (Jensen et al., 2014), whereas larger variations (21.5% to 90.1%) are found for pre-ecal

starch digestion (Meyer et al., 1995). In horses, the pre-ecal starch digestion depends on several factors, such as the type of grain and its characteristics, meal size, and passage rate of digesta (Kienzle, 1994). Further, grain processing

Abbreviations

ADF	acid detergent fiber
AUC	area under the curve
BW	body weight
Cfat	crude fat
CP	crude protein
DG	degree of gelatinization
DM	dry matter
DSC	differential scanning calorimetry
MBT	mobile bag technique
NIR	near-infrared radiation
NDF	neutral detergent fiber
SCFA	short-chain fatty acid
WSC	water-soluble carbohydrates

involving heat and moisture is associated with improving the availability of starch for enzymatic degradation, thereby increasing starch digestion in the small intestine (Svihus et al., 2005). Using the mobile bag technique (MBT), Philippeau et al. (2014) found that pre-cecal starch digestion depended on processing, with the lowest digestion for untreated barley and the highest for ground barley, 55.1% and 97.4%, respectively. Enzymatic starch digestion in the small intestine is preferred, as starch fermentation in the hindgut is associated with a higher concentration of short-chain fatty acids (SCFA) and lactate, decreased pH, and microbial disturbance in equines (Willard et al., 1977; de Fombelle et al., 2003). Therefore, compound feeds and grains used for horses are often processed, and one of the most common processing methods is micronizing (Julliand et al., 2006). It includes thermal heat processing with high temperatures (85 to 125 °C) for a short time using near-infrared radiation (Farrell et al., 2015). Processing methods that include endosperm disruption and heat above 80 °C in combination with moisture will restructure the starch granules, causing gelatinization (Svihus et al., 2005). Gelatinization increases amylolytic degradation because part of the crystalline structure is lost (Svihus et al., 2005). Holm et al. (1988) found that the degree of starch gelatinization and digestion rate in rats to be positively correlated, assuming more starch to be digested and thereby change the metabolic responses, as more glucose will be absorbed in the small intestine. Vervuert et al. (2008) found that thermal processing increased serum glucose and insulin responses when horses were fed extruded barley compared with rolled barley or micronized barley (MB), reflecting a higher digestibility of starch in the small intestine with extrusion than with the other methods. However, from the literature, it is unclear whether the degree of gelatinization (DG) from processing is followed by higher glucose and insulin responses (Vervuert et al., 2003, 2007, 2008). The competition for customers increases the search for other processing methods so that feed producers can achieve a differential product. Toasting is one of the “new” processing methods employed by some equine feed companies. This method is often used in products for human consumption, such as breakfast cereals, flour, and wine (Fares and Menga, 2012; Chira and Teissedre, 2013), primarily to enhance the taste as a result of the Maillard reaction (Martins et al., 2001), and it includes temperatures ranging from 90 to 240 °C (Grala et al., 1994; Mosenthin et al., 2016). Hence, toasting could potentially be as effective as micronizing for improving the small intestine’s digestibility of starch. Nonetheless, to

our knowledge, no study has been conducted on toasting’s effect on nutrient digestibility in horses. Therefore, the objective of this experiment was to compare the effects of micronizing and toasting on starch digestion of barley and maize. It is hypothesized that: 1) toasting is as efficient as micronizing for improving the small intestine’s digestibility of starch; 2) starch digestibility in the small intestine is highly reflected in the blood glucose and insulin responses after feeding, independent of processing method; 3) the amount of starch escaping digestion in the small intestine is low; and 4) fluctuations in cecal pH and SCFA concentrations and proportions after feeding are small, independent of processing method.

Materials and Methods

Experimental design

All housing, management, and experimental procedures followed the laws and regulations for experimental animals in Norway (i.e., Regulations on the Use of Animals in Experiments, July 2015). The experiment was designed as a 4 × 4 Latin square experiment with four experimental periods. Each period consisted of 8 d of diet adaptation followed by 2 d of data collection. Blood and cecal samples for pH and SCFA analyses were collected on day 9, and digestibility in the small intestine was measured on day 10 in each period.

Animals

Four healthy cecum-cannulated Norwegian cold-blooded trotter geldings (age 14 to 26 yr) with an initial body weight (BW ± SEM) of 565 ± 35 kg were used in the experiment. Horses were followed routinely with veterinarian checkups, including vaccinations, dental examinations, and teeth floating. All horses were housed in individual stalls (3 × 3 m) with rubber mats and wood shavings as bedding material. In the adaptation period, horses were allowed access to a gravel paddock for 3 to 4 h/d. In the collection periods, one outdoor visit for 1 h was allowed daily after sampling had ended.

Diets

Treatments were arranged as 2 × 2 factorial, with two processing methods: micronizing and toasting. Two feeds were used: barley and maize. The chemical composition of the feedstuffs is presented in Table 2. Four experimental diets were formulated using hay and processed grains (same batches): MB, toasted barley (TB), micronized maize (MM), and toasted maize (TM) (Table 3). The micronizing and toasting processes are described below. All concentrate was fed once a day at 0600 hours. Seven days prior to the first adaptation period, a mix of the four diets was fed to gradually increase starch intake from 0 to 1 g/kg BW per day. Thereafter, all diets were balanced to provide 1 g starch/kg BW, and the amount of hay was adjusted to a total DM intake of 3 g/kg BW in the meal at 0600 hours. The horses were fed a total of 15.7 ± 0.03 g DM/kg BW per day, which was divided into three meals fed at 0600, 1400, and 2000 hours (Table 3). A commercial supplement of vitamins and minerals (Champion Multitiskud, Felleskjøpet Forutvikling, Trondheim, Norway) and sodium chloride (80 and 25 g/d, respectively) was included with the morning meal. Water was available in the individual stalls’ automatic water troughs and from buckets in the gravel paddock.

Processing

Micronizing and toasting of barley and maize occurred at Felleskjøpet Agri (Skansen, Norway). Approximately 14.5 h prior to the micronizing treatment, the raw maize was preconditioned with water to raise the moisture content to 15.5%. The barley did not receive any preconditioning with water, as it had a moisture content of 11.2%. The barley and maize were then micronized for approximately 45 s at 90 to 105 °C using an infrared micronizer with a heat output of 525 kW (M600/72/HRS, Micronizing Company UK Ltd, Suffolk, UK; Table 1). After micronizing, the heated barley and maize were run through a roller (0.15 mm, TECOM AB, X, Sweden) to produce a flaked product and then cooled down (custom-made cooler; Felleskjøpet Agri, Skansen, Norway). Prior to the toasting treatment (approximately 15 and 12.5 h for maize and barley, respectively), the raw grains were preconditioned with water to raise the moisture content to 20.6% and 22.6% (maize and barley, respectively). Thereafter, the grains were toasted for 30 min at 150 °C (ECOTOAST 600, agrel GmbH agrar Entwicklungslabor, Germany). After toasting, the heated barley and maize were run through a roller (0.35 and 1 mm for barley and maize, respectively; Strukturvalse T80, Vestjysk Smede, Denmark) to produce a flaked product and then cooled down.

Data collection

Feedstuffs

Samples of all feedstuffs were collected regularly during the four data collection periods and stored in sealed plastic bags for later analysis.

Mobile bag technique

The MBT was used to estimate the small intestinal starch digestibility. Bags (1 × 1 × 12 cm) were made from precision-woven open mesh fabric with a porosity of 15 µ (Sefar Nitex, 03-15/10, Sefar AG, Heiden, Switzerland). The bags were prepared by cutting a piece of mesh (large enough for the heat sealing) and folding it in the middle. The mesh was then heat sealed at one end and one side, and then turned inside out to avoid sharp edges. A steel washer (1 cm external diameter, weight 0.3 g) was sealed into the end of each bag, allowing for capture with a magnet in the cecum. Lastly, the bags were marked with a permanent marker for identification. The weights of the bags when empty and when filled with individual feed (1 g/bag) were recorded. All feeds were milled to pass a 1.5-mm screen. The bags (15 bags per horse per period) were soaked in cold tap water before they were placed in the stomach with a nasogastric tube flushed with approximately 1.5 liters of tap water. Bags were administered after feeding half of the morning meal and before feeding hay. The rest of the morning meal and the hay were fed afterward. A string (40 cm long) with a double-sided magnet (approximately 2 cm in diameter) was introduced into the cecum through the cannula to retrieve the bags upon arrival. The bags were removed from the magnet at hourly intervals for

8 h after feeding. Bags not harvested in the cecum were collected in the feces throughout the following days. The capture time of each bag was noted as soon as the bags were collected and, thereafter, hand-rinsed in cold tap water and stored at −20 °C. At the end of the experiment, all bags were thawed at room temperature, washed in cold water for 35 min (Woolprogram, Avantixx 7 Varioperfect, Bosch, Gerlingen-Schillerhöhe, Germany), and then dried at 45 °C for 48 h. The bags were left at room temperature (approximately 25 °C) for equilibration for 24 h prior to weighing. Control bags (4 bags per feedstuff) were soaked for 1 h before washing and drying as described above to determine their nutrient loss. To obtain enough residue for chemical analyses, the collected bags of each feedstuff were pooled to a specific collection time (0 to 3, 4 to 6, and 7 to 9 h), regardless of which horse they came from. All bags found in the feces were pooled for each feedstuff.

Blood samples

Blood samples were collected by jugular vein puncture into 10-mL heparinized tubes (BD Vacutainer, Becton, Dickinson and Company, USA) before the morning meal (time: 0) and hourly thereafter (time: 1 to 8 h). The blood samples were centrifuged (Heraeus labofuge 300, Thermo Fisher Scientific, Waltham, USA) immediately after sampling at 3000 × g for 10 min, and plasma was harvested and stored at −20 °C for later analysis of insulin and glucose concentrations.

SCFA, ammonium nitrogen, and pH

Cecal fluid was collected before the morning meal (time: 0) and thereafter hourly (time: 1 to 8 h). A collection tube and a pH electrode (Sentix 41, WTW, Weilheim, Germany) attached to a data logger (ProfiLine 340i, WTW, Weilheim, Germany) were placed in the cecum according to Jensen et al. (2016) approximately 30 min before the first collection (time: 0). Cecal fluid was sampled (~100 mL) with a 400-mL syringe connected to the tube placed in the cecum. The pH was measured immediately as cecal fluid samples were taken and in situ in the cecum every minute throughout the 8 h time frame with the pH electrode. From this, two subsamples of each 9.5 mL cecal fluid were mixed with 0.5 mL of formic acid and stored at 3 °C for later analysis of SCFA and ammonium nitrogen (N) concentrations.

Chemical analyses

Feed samples from each period were analyzed in duplicate for DM, starch, and crude protein (CP) (Table 2). Samples were milled to pass a 1-mm screen (Cutting mill SM 200, Retsch GmbH, Haan, Germany). For starch, feed samples were milled to pass a 0.5-mm screen before analysis. Dry matter (DM) content was measured by drying to a constant weight (24 h at 105 ± 2 °C), and samples were incinerated at 550 °C for 16 h for ash determination. Starch was measured according to the Association of Official Analytical Chemists (AOAC, method 996.11.) by using heat-stable α-amylase, and water-soluble carbohydrates (WSC) were determined by the

Table 1. Processing conditions for barley and maize

	Toasting				Micronizing			
	Temp. ¹	Duration, min	Heat source	Roller, mm	Temp.	Duration, s	Heat source	Roller, mm
Barley	150	30	Steam	0.35	90 to 105	45	NIR	0.15
Maize	150	30	Steam	1	90 to 105	45	NIR	0.15

¹ Temp, temperature in °C.

Table 2. DM (g/kg), chemical composition (g/kg DM), and DG (%) of hay, micronized maize (MM) or toasted maize (TM), and micronized barley (MB) or toasted barley (TB) (mean $\pm$ SEM)

Nutrient	Hay	MM	TM	MB	TB	P-value ¹	
						F	T
DM	898 $\pm$ 1.46	874 $\pm$ 2.47 ^a	840 $\pm$ 4.27 ^b	881 $\pm$ 1.27 ^A	830 $\pm$ 3.03 ^B	0.338	<0.001
Ash	78.2 $\pm$ 0.85	14.2 $\pm$ 0.31	13.8 $\pm$ 0.65	19.8 $\pm$ 0.12	20.4 $\pm$ 0.30	<0.001	0.862
CP	147 $\pm$ 5.59	86.3 $\pm$ 2.42 ^a	84.2 $\pm$ 1.77 ^b	120 $\pm$ 2.10 ^B	126 $\pm$ 0.71 ^A	<0.001	0.302
CFat	18.6 $\pm$ 1.59	43.4 $\pm$ 3.25 ^a	36.0 $\pm$ 1.10 ^b	14.3 $\pm$ 0.70	15.6 $\pm$ 0.57	<0.001	0.058
Starch	28.9 $\pm$ 0.80	721 $\pm$ 7.89	719 $\pm$ 9.69	601 $\pm$ 5.00	577 $\pm$ 7.88	<0.001	0.145
WSC	84.9 $\pm$ 2.18	27.7 $\pm$ 0.88 ^b	35.4 $\pm$ 1.55 ^a	32.6 $\pm$ 0.50	38.5 $\pm$ 0.60	0.557	0.003
NDF	616 $\pm$ 6.62	95.8 $\pm$ 4.61 ^b	119 $\pm$ 1.30 ^a	224 $\pm$ 2.46	227 $\pm$ 7.32	<0.001	0.051
ADF	341 $\pm$ 4.92	46.7 $\pm$ 0.89	47.8 $\pm$ 1.10	78.6 $\pm$ 0.56	77.1 $\pm$ 1.94	<0.001	0.859
DG		56.8 $\pm$ 1.49	39.1 $\pm$ 3.10	-12.7 $\pm$ 12.0	-34.3 $\pm$ 1.53	<0.001	0.021

¹The effect of feedstuff (F) and treatment (T).^{a,b} or ^{A,B}Values within a row for each feedstuff are different if superscript differs (P < 0.05).**Table 3.** Feed intake (kg) and daily nutrient intake (g/kg BW) for the four diets¹ (mean $\pm$ SEM)

	MM, n = 4	MB, n = 4	TM, n = 4	TB, n = 4
Morning, 0600 hours				
Hay	1.10 $\pm$ 0.03	0.91 $\pm$ 0.03	1.13 $\pm$ 0.04	0.95 $\pm$ 0.03
Supplement	0.88 $\pm$ 0.03	1.05 $\pm$ 0.03	0.90 $\pm$ 0.03	1.10 $\pm$ 0.03
Lunch, 1400 hours				
Hay	3.95 $\pm$ 0.12	3.95 $\pm$ 0.12	3.95 $\pm$ 0.12	3.95 $\pm$ 0.12
Evening, 2000 hours				
Hay	3.95 $\pm$ 0.12	3.95 $\pm$ 0.12	3.95 $\pm$ 0.12	3.95 $\pm$ 0.12
Daily nutrient intake ¹				
DM	15.6 $\pm$ 0.02	15.6 $\pm$ 0.02	15.7 $\pm$ 0.03	15.7 $\pm$ 0.03
Ash	1.13 $\pm$ 0.01	1.12 $\pm$ 0.01	1.14 $\pm$ 0.01	1.13 $\pm$ 0.01
CP	2.21 $\pm$ 0.08	2.25 $\pm$ 0.08	2.22 $\pm$ 0.08	2.27 $\pm$ 0.08
Cfat	0.32 $\pm$ 0.02	0.29 $\pm$ 0.02	0.31 $\pm$ 0.02	0.29 $\pm$ 0.02
Starch	1.39 $\pm$ 0.02	1.39 $\pm$ 0.02	1.37 $\pm$ 0.02	1.34 $\pm$ 0.02
WSC	1.25 $\pm$ 0.03	1.25 $\pm$ 0.03	1.27 $\pm$ 0.03	1.26 $\pm$ 0.03
NDF	8.91 $\pm$ 0.09	8.97 $\pm$ 0.09	8.97 $\pm$ 0.09	9.01 $\pm$ 0.09
ADF	4.92 $\pm$ 0.08	4.89 $\pm$ 0.08	4.94 $\pm$ 0.08	4.91 $\pm$ 0.08

¹MM, micronized maize; TM, toasted maize; MB, micronized barley; TB, toasted barley.

method described in the study of [Randby et al. \(2010\)](#). Nitrogen was determined according to the Dumas method (Elementar Analysensysteme GmbH, Hanau, Germany), and CP was calculated as N $\times$ 6.25. Crude fat was analyzed according to the accelerated solvent extractor method (Dionex ASE 350, Thermo Fisher Scientific, Waltham, USA). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using the filter bag technique described by [ANKOM \(2017a, 2017b\)](#). Residues from the mobile bags were analyzed for starch and N as described above. Plasma glucose was analyzed by the hexokinase method according to [Tietz et al. \(1995\)](#), and insulin was analyzed using the ELISA test (Mercodia AB, Uppsala, Sweden). Cecal fluid was analyzed for the concentration of SCFA (times: 0, 1, 3, 5, and 7 h) and ammonium N (times: 0 and 3 h). The concentrations of SCFA were determined by gas chromatography (Trace 1300 GC, Thermo Fisher Scientific, Waltham, USA), and ammonium N was measured according to [AOAC International \(2002\)](#) method 2001.11, besides the first digestion step. The DG was evaluated using the differential scanning calorimetry (DSC) method. The DSC method relies on the enthalpy measurement of non-processed and processed samples, and the difference between the two represents the extent of gelatinization with a greater

difference indicating greater gelatinization. A DM feed sample weighing approximately 30 mg (ground through a 0.5-mm screen) was weighed in a stainless-steel pan, and deionized water (1:2, feed/water, wt/wt, total weight 90 mg) was added. Thermal scans were conducted using a differential scanning calorimeter (DSC 823, Mettler Toledo, Stockholm, Sweden). The measurement was performed by heating the pan in the DSC from 10 to 120 °C at a heating rate of 10 °C/min. The onset, peak, and conclusion gelatinization temperatures and the enthalpy of gelatinization (ΔH) were then determined. The DG is calculated as $DG (\%) = [(\Delta H_0 - \Delta H_1) / \Delta H_0] \times 100$, in which ΔH_0 is the gelatinization enthalpy of starch (J/g starch) in a non-processed sample and ΔH_1 is the gelatinization enthalpy of starch in a processed sample (J/g starch). A 100% DG equates to completely processed starch, whereas 0% equates to unprocessed starch, and negative values indicate lower DG in the processed sample than the non-processed sample. All measurements were performed in duplicate.

Statistical analyses

All statistical analyses were performed in Rstudio (version 1.1.456, Rstudio Inc., Boston, USA). Analysis of variance was done on the

chemical composition of the feedstuffs with a model comprising nutrient as response and feed and treatment as predictors. The DM, starch, and CP disappearance were subjected to ANOVA, with the nutrient disappearance as response and feed, and treatment and time (DM) or time interval (starch and CP) and their interactions as predictors. Mean concentrations, peak concentration, time to peak, and number of peaks were calculated for plasma glucose and insulin. Calculations of area under the curve (AUC) above baseline (without considering area beneath) were performed for glucose and insulin in GraphPad Prism (version 8.0.1, GraphPad Software, San Diego, USA), and ANOVAs were performed in a model comprising either mean concentration, peak concentration, time to peak, or number of peaks and AUC as response, with feed, treatment, and their interactions (if present) as predictors. Analyses of SCFA, ammonium N concentrations, and pH were performed using mixed models for repeated measurements. The model comprised the fixed effect of feed (barley or maize), treatment (micronizing or toasting), time (after feeding), interaction (feed $\times$ treatment), and the random effect of horse. Significant differences of least-square means were analyzed by Tukey's Honest Significant Difference test (Rstudio, version 1.1.456, Rstudio Inc., Boston, USA). All results are presented as least-square means with SEM as a measure of variance. Effects are considered significantly different if $P < 0.05$ and a tendency if $P < 0.10$.

Results

All horses remained healthy and in good condition throughout the experiment. Residues from the previous evening meal were collected for two horses on the day of sampling (one horse in period 3:1.6 kg DM and two horses in period 3:0.7 and 1 kg DM, respectively). The residue was offered to the horses and eaten after sampling had ended.

Chemical composition of the feedstuffs

The chemical composition of the feedstuffs is presented in Table 2. Hay has the highest numerical DM content compared with maize and barley. An effect of treatment ($P < 0.001$) was measured for DM, with micronizing having the highest content for both maize and barley. Barley had the highest content of CP ($P < 0.001$) compared with maize. Toasting had the highest ($P = 0.003$) WSC content for both barley and maize. The starch content was highest in maize compared with barley ($P < 0.001$), whereas hay had the lowest numerical content. Crude fat was highest in maize compared with barley ($P < 0.001$). NDF and ADF were highest in barley compared with maize ($P < 0.001$). The DG was highest for MM compared with the other diets (Table 2). However, DG for processed barley was negative, indicating that processed barley had a lower DG than whole barley. The negative DG for barley was interpreted as zero DG for barley. The DM intake for each meal and daily nutrient intake is shown in Table 3. The size of the grain meal within each diet varied to ensure similar starch intake.

Nutrient disappearance

The DM loss from the control bags was $7.3 \pm 1.4\%$, $9.9 \pm 1.9\%$, $6.5 \pm 1.6\%$, and $9.6 \pm 1.0\%$ for MM, TM, MB, and TB, respectively. The effects of feed, treatment, time, and their interactions on DM, starch, and CP pre-cecal disappearance are shown in Figure 1. There was an effect of the interaction, feed $\times$ treatment $\times$ time ($P < 0.001$), and the DM disappearance from the mobile bags increased over time; it was at all times highest for the MM compared with the other feedstuffs. Starch disappearance

increased with later time intervals, and an interaction between feed $\times$ treatment ($P = 0.048$) was measured with maize and micronizing having the highest disappearances compared with barley and toasting. Disappearance of CP increased over time ($P = 0.041$), regardless of feed or treatment.

Metabolic response in plasma

The effects of feed, treatment, and their interaction on plasma glucose and insulin measurements are presented in Table 4. Treatment did not affect any of the measured variables for plasma glucose and insulin. Feed had no effect on the measured variables for plasma insulin. There was no effect of feed on peak and the number of peaks for plasma glucose. However, plasma glucose peaked later ($P = 0.045$) for maize than for barley. Regarding AUC, an interaction between feed and treatment was found for glucose ($P = 0.015$), with a larger AUC for TB than for MB and MM or TM.

Digestive response in the cecum

The effects of feed, treatment, time, and their interactions on SCFA concentrations and molar proportions are shown in Figure 2. The concentration of total SCFA increased after feeding ($P < 0.001$), with a higher concentration for barley than for maize ($P = 0.044$; Figure 2a). Generally, the molar proportion of acetate was the greatest, followed by propionate and then butyrate for all diets at all time points. However, the molar proportion of acetate ($P = 0.004$) first increased and then decreased with time (Figure 2b), whereas the opposite was found for propionate ($P = 0.006$; Figure 2c). Firstly, the proportion of butyrate ($P = 0.086$) tended to increase and thereafter decrease with time (Figure 2d), whereas iso-butyrate ($P < 0.001$; Figure 2e) and iso-valerate ($P < 0.001$; Figure 2g) decreased after feeding. Further, butyrate tended to be higher ($P = 0.058$) for micronizing than for toasting (Figure 2d). An interaction between feed and time ($P < 0.001$) was present for valerate, as the proportion after feeding increased for barley; however, maize remained the same (Figure 2f). The (C2 + C4)/C3 ratio ($P = 0.055$) tended to first increase and then decrease after feeding, reflecting the changes in molar proportions of acetate, propionate, and butyrate over time (Figure 2h). No effects of feed, treatment, or their interaction were found on ammonium N. But the mean concentrations of ammonium N decreased over time ($P < 0.001$), with MM from 57.5 to 23.2 mg/L, MB from 65.7 to 22.3 mg/L, TM from 65.9 to 17.2 mg/L, and TB from 65.8 to 19.5 mg/L. The pH decreased after feeding, reaching a minimum pH after 195, 173, 180, and 150 min for MM, MB, TM, and TB, respectively (Figure 3). The pH then fluctuated before increasing again. Feed, treatment, and their interaction had no effect on cecal pH.

Discussion

Starch digestion has been previously investigated in horses using different direct and indirect methodologies. Small intestinal cannulated horses (Meyer et al., 1995), slaughter experiments (de Fombelle et al., 2003), and the MBT (Philippeau et al., 2014) have been used as more direct methods for quantifying starch digestion in different segments of the gastrointestinal tract of horses. Blood glucose and insulin responses (Healy et al., 1995; Vervuert et al., 2004, 2007; Jensen et al., 2016) and changes in fermentation parameters in the cecum (McLean et al., 2000) of horses have been used as a proxy to evaluate the degree of starch digestion in the small intestine and cecum, respectively. However, the results have been inconclusive. To the authors' knowledge, this is the first

Figure 1. DM, starch, and CP pre-ecal disappearance for each of the four diets (MM, MB, TM, and TB) for each hour or time interval (1 = 0 to 3 h, 2 = 4 to 6 h, and 3 = 7 to 9 h), respectively. Differences are given for feed (F), treatment (T), and time/time interval (Ti) and interactions.

study to include both metabolic responses in blood and the digestive responses in cecum in combination with results from the MBT. The results presented here show the complexity of evaluating starch digestion in horses by only including one of the above-mentioned methodologies.

Pre-ecal disappearances of starch and protein

It is assumed that nutrients lost from mobile bags harvested in the cecum are digested in the small intestine. In the present study, the pre-ecal disappearance of starch and protein varied from 55% to 81% and 82% to 95%, respectively. This is in accordance with previous studies using the MBT (Hymøller et al., 2012; Philippeau et al., 2014). Protein digestion was relatively high and not affected by processing, while high starch digestibility was expected due to the maize and barley being processed. However, some variation was measured in the starch disappearance. In the present study, the average starch

intake was 565 g/d, and according to MBT, starch measurements of approximately 107, 164, 122, and 254 g/d escaped digestion in the small intestine for MM, MB, TM, and TB diets, respectively. Since the apparent total tract digestibility of starch in grains is found to be nearly 100% (Jensen et al., 2014), it is expected that the undigested starch was fermented mainly in the hindgut. Some starch might be fermented by gastric microbiota present in the saccus cecus in the nonglandular region of the stomach (Coenen et al., 2006; Varlout et al., 2007). However, to what extent starch is fermented in the stomach still needs to be quantified. The site of starch digestion in the gastrointestinal tract of the horse (pre-ecal or hindgut) is expected to influence the metabolic responses, as discussed below.

Metabolic response in plasma

In the present study, it was hypothesized that starch digestion in the small intestine was reflected in the blood glucose and

Table 4. Mean $\pm$ SEM peak (ng/L), time to peak (h), and AUC (ng $\times$ h/L) for glucose (G) and insulin (I) with different diets

Feed treatment ¹						P-value ²		
		MB	TB	MM	TM	F	T	F $\times$ T
Peak	G	5.88 $\pm$ 0.13	5.85 $\pm$ 0.18	5.85 $\pm$ 0.19	5.78 $\pm$ 0.23	0.794	0.794	0.794
	I	386 $\pm$ 56.8	354 $\pm$ 26.5	460 $\pm$ 64.7	394 $\pm$ 65.0	0.325	0.397	0.765
No. of peaks	G	1.75 $\pm$ 0.48	1.25 $\pm$ 0.25	1.25 $\pm$ 0.25	1.50 $\pm$ 0.29	0.712	0.712	0.279
	I	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00			
Peak time	G	1.00 $\pm$ 0.00 ^b	1.25 $\pm$ 0.25 ^b	1.50 $\pm$ 0.29 ^a	2.00 $\pm$ 0.41 ^a	0.045	0.205	0.663
	I	1.25 $\pm$ 0.25	1.25 $\pm$ 0.25	1.00 $\pm$ 0.00	1.25 $\pm$ 0.25	0.574	0.574	0.574
AUC	G	2.32 $\pm$ 0.28 ^{ab}	3.48 $\pm$ 0.44 ^a	2.89 $\pm$ 0.57 ^{ab}	1.75 $\pm$ 0.25 ^b	0.177	0.983	0.015
	I	1,373 $\pm$ 156	1,433 $\pm$ 74.9	1,444 $\pm$ 119	1,220 $\pm$ 112	0.562	0.502	0.256

¹MM, micronized maize; TM, toasted maize; MB, micronized barley; TB, toasted barley.

²The effect of feedstuff (F), treatment (T), and their interaction (F $\times$ T).

^{a,b}Values within a row are different if superscript differs (P < 0.05).

insulin responses after feeding, independent of the processing method. This was the case, as both plasma glucose and insulin increased after feeding. This was also measured in earlier studies (Vervuert et al., 2003, 2004, 2009). In the present study, MM had a higher pre-cecal DM and starch disappearance from mobile bags compared with the other diets, but no differences were found between feeds or treatments for either plasma glucose or insulin. Similar findings for whole vs. thermally processed barley on starch disappearance and glucose and insulin responses were measured by Philippeau et al. (2014). This contradicts the theory that increased starch digestibility should increase the glucose concentration in the blood and further increase the insulin response (Palumbo et al., 2013). Yet, it is unclear to what degree the disappeared starch from MM was enzymatically digested or possibly degraded by microbes, as they are present along the entire gastrointestinal tract including the stomach (de Fombelle et al., 2003).

The AUC is often used as a parameter to describe both the overall plasma glucose and insulin responses after feeding. However, contradicting results are found for grain processing on AUC. Vervuert et al. (2003) and Vervuert et al. (2004) did not measure any effect of processing oats or maize (untreated vs. thermal processing) on glucose or insulin AUC, respectively. Yet, Vervuert et al. (2008) measured a larger glucose AUC for extruded compared with rolled barley and MB, along with a larger insulin AUC for extruded and MB compared with rolled barley. In the present study, an interaction between feed $\times$ treatment was found for AUC, with TB having a higher AUC for glucose compared with MB, MM, and TM. TB peaked twice during the sampling time, whereas MB, MM, and TM only peaked once. The time for peaks to occur and the number of peaks could indicate the differences in gastric contractions and thereby gastric emptying. Lorenzo-Figueras et al. (2005) describe gastric emptying as a combination of relaxation of the proximal portion of the stomach, suppression of antral motility, and stimulation of the pyloric contractions, all working together at once. The composition of the meal combined with volume, physical structure, energy density, and osmolarity can affect the rate of gastric emptying (Meyer et al., 1986). Slower gastric emptying is measured with a starch-rich meal (1.25 g starch/kg BW) compared with a meal low in starch (0.66 g starch/kg BW; Métayer et al., 2004). However, in the present study, all meals were similar in starch content. Yet, plasma glucose peaked later for maize than for barley. In general, meals containing maize were smaller in volume compared with those containing barley, as the starch content

was higher in maize than barley; thereby, less was required to obtain 1 g starch/kg BW per meal. This contradicts smaller meals resulting in faster gastric emptying compared with larger meals (Métayer et al., 2004). On the other hand, the difference in meal size is small in the present study, and the effect on gastric emptying may have been limited. Another approach could be physical structure, osmolarity, or even the ratio between amylose and amylopectin in the grains. In general, maize has a higher swelling- and water-binding capacity than barley (Brøkner et al., 2012). This suggests a higher ratio of amylopectin to amylose, as it is easier to solubilize (Cowieson et al., 2019). Furthermore, Hymøller et al. (2012) measured a longer average pre-cecal passage time of mobile bags containing soaked maize compared with soaked barley (7.99 and 6.82 h, respectively), supporting the theory of why plasma glucose peaked later for maize than for barley. Maize and barley contain approximately similar ratios between amylose and amylopectin (approximately 25% and 75%, respectively; Svihus et al., 2005; Cowieson et al., 2019), but it cannot be excluded that maize had a higher amylopectin ratio, as it was not measured in the present study.

Digestive response in the cecum

In general, plasma glucose and insulin concentrations are parameters of pre-cecal digestion, whereas the cecal SCFA concentration together with pH gives an indication of fermentation in the hindgut of the horse. Further, the time to reach maximum SCFA concentration and minimum pH in cecum can indicate the passage rate of the feed from the stomach to the cecum and the fermentability of the escaped starch. In the present study, SCFA concentrations increased relatively fast after feeding (approximately 1 to 2 h), and maximum SCFA concentrations were measured approximately 3 h after feeding. Jensen et al. (2016) measured both an increase in SCFA concentration and a corresponding pH drop approximately 3 h after feeding horses a pelleted barley meal (2 g starch/kg BW). In the present study, barley had a higher total SCFA concentration compared with maize, with TB having the highest SCFA concentration, and, furthermore, a lower pre-cecal starch disappearance up to 6 h after administration, reflecting starch being fermented in the cecum. The proportions of acetate and propionate also indicate the fermentation of starch. McLean et al. (2000) measured higher lactate and total SCFA with both higher acetate and propionate concentrations and lower cecal pH 4 to 8 h after feeding rolled barley compared with micronized and extruded barley, indicating that less starch reached the

Figure 2. Concentration of SCFA (mmol/L) and molar proportions (%) measured hourly (mean $\pm$ SEM) in the cecal fluid after feeding MM, TM, MB, and TB. Differences are given for feed (F), treatment (T), and time (Ti) and interactions.

cecum when using these processing techniques compared with rolling. Similar results are measured for propionate, lactate, and pH by increasing rolled barley in the ration, thereby increasing

daily starch intake (Julliand et al., 2001). Starch intake was approximately 2 g/kg BW per meal in the studies by Julliand et al. (2001), McLean et al. (2000), and Jensen et al. (2016), and the

Figure 3. pH fluctuations in cecum measured in 30-min intervals for the average of the four diets after feeding MM, TM, MB, and TB. Differences are given for feed (F), treatment (T), and time (Ti).

minimum pH varied from 6.26 to 6.40, which is lower than the minimum pH in the present study. When feeding either starch at approximately 2 g/kg BW per meal or hay-only diets, cecal pH varied from 6.26 to 6.40 and 6.50 to 6.74, respectively (McLean et al., 2000; Julliand et al., 2001; Jensen et al., 2016). In this study, the decrease in cecal pH was in between the above studies. Altogether, this indicates that processed starch meals fed at a level of 1 g/kg BW can to some extent escape the enzymatic digestion in the small intestine, thereby interfering with the microbiota and concentrations and ratios of SCFA and pH.

In this study, it is possible that the processing methods that included thermal heat increased the pre-cecal starch digestibility as a result of an increased DG. When comparing the DG in the present study, no gelatinization occurred for either of the two barley diets. Whereas, for maize, micronizing had a larger impact on DG compared with toasting. Vervuert et al. (2004) also measured an increased DG when maize was micronized compared with untreated maize. In general, maize has a higher gelatinization enthalpy, meaning that lower temperatures and moisture content are required to gelatinize maize starch compared with barley starch (Tan et al., 2008). However, both Vervuert et al. (2007) and Philippeau et al. (2014) measured the effect of processing barley on DG. From these two studies, ground barley had a DG varying from 15% to 18%, indicating a possibility of a lower DG for TB and MB in the present study. Yet, Rosenfeld and Austbø (2009) did not measure an effect of micronizing grains on pre-cecal starch disappearance as in the present study. An in vitro study demonstrated lower starch digestibility of peas when toasted compared with being extruded and expanded (Masoero et al., 2005). This is also confirmed in pigs, where a lower ileal starch digestibility of toasted peas compared with dried was measured (Canibe and Knudsen, 1997). However, it can be difficult to compare results across studies, as the processing conditions (moisture content, duration, temperature, and pressure) vary.

Methodical and practical recommendations

In summary, the results presented here show the complexity of evaluating starch digestion in horses. Future studies should include detailed information regarding processing (duration, temperature, moisture content, pressure, and machinery), diet characteristics (composition and DG), and feeding management (g/kg BW per meal, number of meals, and feeding order of hay and concentrate), as well as information regarding the techniques used to study starch digestion. This would provide a better basis for comparing and interpreting results.

From a practical point, the results presented in this study indicate that processing affected the DG in maize more than in barley. Furthermore, compared with toasting, the preferred processing technique for improving the starch digestion based on the disappearance of starch from the mobile bags is micronizing. The metabolic responses in plasma and digestive responses in the cecum revealed more of a change over time than an effect of processing and type of grain on the measured variables. However, the SCFA concentration was highest in the TB compared with the MB, TM, and MM, supporting the lower digestibility of starch in the small intestine from the TB. The effect of the changes measured in the cecum in this study on the hindgut health can be questioned. Whereas, the energy value of starch is lower when fermented to SCFA than with enzymatical digestion in the small intestine with the absorption of glucose. The results from this study revealed that when feeding only 1 g processed starch/kg BW per meal, starch escapes the enzymatic digestion in the small intestine, and there is still a lack in our knowledge regarding the diet effects on gastric emptying and passage rate through the small intestine for improving enzymatical starch digestion.

Conclusions

In the present study, it was hypothesized that toasting was as efficient as micronizing to improve starch digestibility. However, this was not the case when evaluating the small intestinal digestibility of starch. Therefore, to increase the pre-cecal starch digestibility, the preferred processing method is micronizing for both barley and maize when measured by the MBT. Further, starch digestibility for both barley and maize was highly reflected in the metabolic responses of plasma glucose and insulin after feeding, but no effect of processing method was measured. Fluctuations in both cecal pH and SCFA concentrations after feeding were significant, and the starch escaping the enzymatical digestion in the small intestine was higher than expected.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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