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Original Research

Effect of micronization and meal size of corn grain on glycemic response and *in vitro* hindgut acidosis potential in horses

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ABSTRACT

This study aimed to 1) evaluate the interaction of corn grain micronization and starch levels per meal on equine plasma glucose, and 2) determine if micronization affects the risk of hindgut acidosis. Six mature (aged 6 to 10 years), healthy, non-pregnant mares (initial body weight [BW]: 301 to 463 kg) were used in a 2×3 factorial cross-over design. The treatments included two forms of corn grain (ground and micronized flaked) at three levels of starch (1, 1.5, and 2 g/kg BW per meal). The blood was sampled before and 30, 60, 90, 120, 180, 240, and 300 min after morning feeding and the glucose concentration in the plasma was determined. Small intestine and hindgut dry matter (DM) disappearances of ground and micronized corn were also compared using *in vitro* techniques. Micronized flaked corn grain showed three times more *in vitro* enzymatic DM disappearance ($p < 0.001$) compared with ground corn. Residues of *in vitro* enzymatic digestion of micronized flaked corn fermented 38.59 % faster than ground corn during *in vitro* hindgut incubation. The horses that consumed micronized flaked corn had higher post-prandial plasma glucose concentrations ($p < 0.001$). Increasing starch levels per meal from 1–2 g/kg BW resulted in higher plasma glucose concentrations ($P = 0.005$). However, no interaction of processing and starch meal size was found. Overall, processing the corn grain by micronization or increasing starch level per meal increased the plasma glucose concentrations, but the magnitude of the increases did not match that expected from *in vitro* studies.

1. Introduction

The small intestinal digestibility of cereal starch depends on the type of grains [1]. Oat has a relatively greater small intestinal starch digestibility which makes it the most suitable grain for feeding to horses [2]. Other grains, especially corn, are less digestible despite their higher starch content [3]. Large meals containing starch may not be fully digested in the horse's small intestine due to the limited retention time in this section of the digestive tract and the inadequate amount of amylase enzyme [4]. Therefore, if horses are fed large meals containing poorly digested starch, significant amounts of undigested starch may reach the hindgut [5]. As a result, starch escaping enzymatic digestion in the foregut is anaerobically fermented in the large intestine which may cause chronic or acute hindgut acidosis, a metabolic disorder that harms animal health [6]. Julliand et al. [4] determined a safe starch level of 2 g/kg body weight (BW) per meal. Vervuert et al. [7] and Coenen and Vervuert [8] reduced this level to just l g/kg BW per meal. Additionally, Thorringer et al. [9] found that the amount of starch that escaped enzymatic digestion in the small intestine was higher than expected.

Heat treatment is an effective solution to increase the enzymatic digestion capacity of starch in the small intestine of horses, which could allow for less starch reaching the hindgut. Micronization is a heat treatment in which materials such as cereals, legumes, oilseeds, etc. are processed using infrared radiation in a short time and at high temperatures [10]. This processing method has the potential to increase the digestible energy of grains for horses [9,11]. Starch utilization from heat-processed grain depends on the type and variety of grain, amount and duration of heating, final physical form, and particle size that are provided to the animal. In other words, thermal processing does not guarantee a higher energy value of heat-processed grain compared to ground grain [12]. Flaking is a post-micronization process that affects starch gelatinization [10]. However, in the literature, it is rarely specified if grains were ground or flaked after micronization [11]. Furthermore, flake density affects enzymatic starch accessibility [13], but this property was not reported in previous studies using micronized-flaked corn and barley grains [9,14]. Therefore, full processing specifications

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should be reported in studies examining the nutritional advantages of micronized-flaked corn grain.

Processed grains should be less likely to reach the hindgut and so have a lower hindgut acidosis risk compared with unprocessed grains, especially when heat-treated grains are fed at lower volumes per meal. However, while *in vitro* experiments have shown increased enzymatic digestion of cereal starch as a result of heat treatment, some *in vivo* equine studies did not show improvements in glycemic response [9]. Moreover, there is no evidence that thermal processing of grains guarantees greater starch levels/meal in the horses. Therefore, the objectives of this study were to (1) evaluate the interaction of corn grain micronization (with specific flake density) and starch meal sizes on equine plasma glucose, and (2) determine if micronization affects the risk of hindgut acidosis.

2. Materials and methods

2.1. Corn grain processing

Corn grain samples in this experiment, which were imported from Brazil, were sieved by a 4 mm mesh, soaked with 10 % tap water, and stored at room temperature (25 ± 2 ˚C) for one hour in a plastic container. Tempered seeds were exposed to infrared radiation for 60 s in a commercial micronizer machine (Faravardaneh Ferdowsi Mashhad company, Ltd. Mashhad, Iran) and immediately flaked after exiting the micronizer using a flaker machine (Faravardaneh Ferdowsi Mashhad company, Ltd. Mashhad, Iran) equipped with two rollers with a 1 mm gap. Corn grain from the same batch was milled with a hammer mill (5 mm mesh, Toos Shekan, Mashhad, Iran) and was used as a control (ground corn grain).

2.2. Measurement of water absorption

Micronized flaked corn samples were ground with a similar hammer mill to the one used for grinding corn grains and were used to measure water absorption. The water absorption index (WAI) of the ground control and ground micronized flaked corn was measured according to the method described by Zarzycki et al. [15] with some modifications. In brief, 2 g of each treatment were poured into pre-weighed nylon bags (SEFAR, Switzerland) with 23 μm pore size and placed in 50 ml conical centrifuge tubes. Thirty mL of distilled water was added to the conical centrifuge tubes containing the sample and kept stationary at room temperature for 15 min. Un-absorbed water was drained from each tube within 20 min and WAI was calculated using the following formula:

$$\mathrm{WAI}\,(\%) = (\mathrm{Wg}/\mathrm{WDM}) \times 100 \quad (1)$$

Where:
Wg: weight of dry matter (DM) of sample plus absorbed water.
WDM: weight of DM of the original sample before soaking

2.3. Density and particle size of processed grains

The densities of micronized flaked and ground samples were measured using the procedure described by Schwandt et al. [13] as the ratio of the mass of grain to its bulk volume. The particle size distribution of the samples was measured in triplicates by dry sieving of 100 g representative samples through sieves of 4, 2, 1, 500, 250, 125, 63 and 45 μm for 10 min using a vibratory sieve shaker (Restch AS 200, Germany). The amount of materials retained on each screen size was then determined, and the geometric mean diameter (GMD) and geometry standard deviation (GSD) of the sample were calculated as described by Amerah et al. [16].

2.4. In vivo experiment

2.4.1. Animals and feeding

The *in vivo* experiment of the current study was conducted in the Radan horse club, Hashtgerd, Iran, and other laboratory works and *in vitro* experiments were performed in the central laboratory of the Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran. All the experimental procedures and animal manipulations were approved by the Animal Care and Use Committee of Ferdowsi University of Mashhad (FUM; approval number 526/31/10/2021) outlined by the Iranian Council of Animal Care [17]. Furthermore, the Ethics Committee of FUM accepted and supervised all of the experimental processes.

Six mature (aged 6 to 10 years), healthy, non-pregnant mares (initial BW: 301 to 463 kg), were used in a 2 × 3 factorial cross-over design. The treatments included two forms of corn grain (ground and micronized flaked) which were used at three levels per meal to provide 1, 1.5, and 2 g of starch per kg BW per meal. Horses were housed individually over the duration the experimental period and randomly assigned to receive one of the six treatments on each sampling day. Animals had free access to fresh water throughout the experiment. During non-sampling days (Fig. 1), the horses were fed medium-quality alfalfa hay (DM = 875, crude protein = 175, crude fiber = 290, ether extract = 22 and ash = 109 g/kg, DM basis) twice daily at 2 % BW (DM basis) and a fixed amount (1 kg) of a commercial texturized concentrate mixture once a day. The concentrate mixture (DM = 884, crude protein = 175, crude fiber = 80, ether extract = 52 and ash = 72 g/kg, DM basis) was composed of micronized flaked barely, micronized flaked corn, micronized flaked canola seed and protein pellet at the ratio of 350, 330, 50 and 270 g/kg DM, respectively. The protein pellet had a 6 mm diameter and contained soybean meal, wheat bran, sugar beet pulp, barley grain, calcium carbonate, monocalcium phosphate, salt, mineral & vitamin supplement, glumatine, bentonite at 560,170,80,70,10,10,20,30,30,10 and 10 g/kg DM, respectively. On each blood sampling day, horses were fed only experimental treatments instead of the concentrate meal (Fig. 1). After the last blood sampling, horses were fed half of their daily alfalfa hay and the remaining portion was offered at the night meal. There were two non-sampling days between each sampling day.

2.4.2. Blood collection and analysis

Jugular blood (30 ml) was sampled via venipuncture before and 30, 60, 90, 120, 180, 240, and 300 min after morning feeding at 07:00 h. Blood was collected into an evacuated tube containing potassium oxalate and sodium fluoride as anti-coagulant and anti-glycolytic agents, respectively. Samples were maintained on ice until centrifuged for 10 min at 1059 g. Plasma was obtained and frozen at –20 ˚C. The glucose concentration was determined in duplicate with glucose kit (Farsa Med Parsiyan Company, Tehran, Iran) using an auto-analyzer (BT1500, Rome, Italy). Areas under the curves (AUC) of plasma glucose response were calculated using GraphPad Prism 6 software.

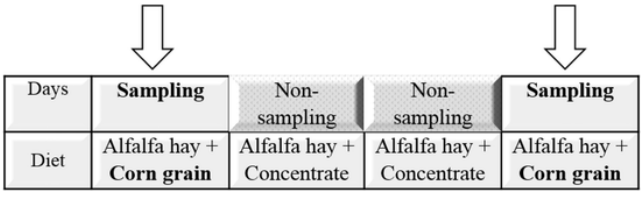

Fig. 1. The feeding program during experimental period. Six horses were tested for each of the experimental conditions (1, 1.5 or 2 g starch /kg BW from micronized versus ground corn) and there were six sampling days to obtain six replicates for each of the treatments.

2.5. In vitro enzymatic digestion

A method previously described by Ngonyamo-Majee et al. [18] was used to simulate enzymatic DM digestion of corn grain in the foregut with small modifications. Ground and micronized flaked corn grains were used in their original particle size as they were fed to the horses in the animal trial with seven replicates for each substrate. Two grams of substrate and 40 ml of 0.1 N HCL solution containing 1 g per liter of pepsin with pH 1.9 were added to 250 ml bottles. The bottles were then incubated at 37 ˚C for one hour in a shaker incubator (D-38678 Clausthal-Zellerfeld). At the end of this step, 2 ml of 0.1 normal sodium hydroxide and 54 ml of pancreatic phosphate buffer solution were added to each bottle. The bottles were then incubated for 6 h to measure the enzymatic DM disappearance. The residuals were filtered using a nylon cloth with 23 μm pores and were placed in an oven at 60 ˚C for 24 h. Next, the percentage of DM disappearance was calculated based on the weight of the samples before and after enzymatic digestion.

2.6. In vitro gas production technique

In vitro gas production of residual DM obtained from enzymatic digestion was carried out based on the method proposed by Menke and Steingass [19] with some modifications. For this experiment, 0.5 g of the residuals from enzymatic digestion were weighed in 250 ml glass bottles. Fresh feces were collected from the rectum of two horses (450 kg BW) which were fed alfalfa hay and concentrate mixture at a ratio of 80:20 at 2.5 % of their BW. Feces were transferred into an insulated double-layered plastic bag filled with CO_2 and immediately transferred to the laboratory under anaerobic condition to be used as an inocula source.

Eight hundred and twenty-five grams of feces were mixed with 3 L anaerobic media of Menke and Steingass [19] in a warmed glass jar (39 ˚C) and filtered using four layers of cheesecloth under continuous flushing of carbon dioxide [20]. The bottles were filled with 75 mL of the buffered fecal liquor and tightly sealed with screw plastic caps. Incubation was performed in a water bath at a temperature of 39 ˚C for 24 h and gas production was measured using an automated gas pressure recording device as described in previous work [21]. In this process, three additional bottles without substrate were used as blanks to correct digestibility calculations. The following generalized Michaelis-Menten model without a lag phase [22] was used for fitting gas production data (without correction for blank):

$$GP = A \times \left(T^n / \left(T^n + K^n\right)\right) \quad (2)$$

where GP is gas production at time T, A is the asymptote gas production (mL), n is the value determining the shape of the curve and k is the time to produce half of A.

After 24 h and immediately after the termination of incubation, the bottles were removed from the machine and placed in cold water to stop the fermentation process. The final pH in the culture liquid was measured using a pH meter (Metrohm 691). The contents of each bottle were then filtered using nylon cloths (23 μm pore size). Solid residues after filtration were transferred to the plastic tubes and dried in an oven at 60 ˚C for 48 h. The dried materials were weighed and subtracted from the blank and used for the calculation of *in vitro* hindgut DM digestibility.

2.7. Statistical analysis

Plasma glucose data was analyzed using the repeated measures MIXED procedure by SAS software, version 9.4 (SAS Institute Inc., Cary, NC). A 3 × 2 factorial randomized cross-over design with the following statistical model was used in this study:

$$Y_{igjkl} = \mu + A_i + D_g + P_j + I_k + T_l + \left(P_j \times T_l\right) + \left(I_k \times T_l\right) + \left(P_j \times I_k\right) + \left(P_j \times I_k \times T_l\right) + e_{igjkl} \quad (3)$$

where Y_{igjkl} is the dependent variable; μ is the overall population mean; A_i is the random effect of the horse; D_g is the random effect of the day of sampling; P_j is the fixed effect of processing; I_k is the fixed effect of feeding level; T_l is the fixed effect of the time; $(P_j \times T_l)$ is the interaction between processing and time; $(I_k \times T_l)$ is the interaction between feeding level and the time; $(P_j \times I_k)$ is the interaction between the processing and intake level; $(P_j \times I_k \times T_l)$ is the tripartite effect of processing, intake level and time and e_{igjkl} is the random residual error term (assumed to be random and independently distributed). For the AUC, the effect of the time (T_l) was omitted from the above model. Other data (corn grain physical properties, *in vitro* hindgut/enzymatic DM disappearances and gas production kinetic parameters) were analyzed by using the general linear model procedure (SAS Inst. Inc., Cary, NC) as a completely randomized design. Data are presented as least squares means ± SEM. Results were considered significant at $P \leq 0.05$; trends were observed at $P \leq 0.10$. The Tukey method was used to test pairwise differences.

3. Results

3.1. Corn grains analysis

Physical properties, particle size distribution and starch content of ground and micronized flaked corn grains are summarized in Table 1. Micronization increased DM content ($p < 0.001$) and water absorption index ($P = 0.003$). However, starch content was not affected by the type of processing. The apparent density of ground corn grain was reduced as a result of micronization and flaking ($P = 0.007$) and the GMD of the ground corn was smaller than ($p < 0.001$) micronized flaked. The GSD of ground and micronized flaked corn were also different ($p < 0.001$).

3.2. Plasma glucose responses

The effect of processing on the horses' plasma glucose concentration at different times after feeding three starch levels per meal supplied by corn is shown in Fig. 2. Fig. 3 also shows the overall effect of processing

Table 1
Physical properties, particle size distribution and starch content of ground and micronized flaked corn grains.

Items	Corn grains		SEM	P value
	Ground	Micronized flaked		
Dry matter (g/kg as fed)	891.70	907.50	1.72	< 0.001
Starch (g/kg DM)	700.12	701.52	1.56	0.63
Apparent density[1] (kg/L)	0.58	0.32	0.09	0.007
Water absorption index (%)	253.10	317.70	2.20	0.003
Particle size distribution[2]				
4000 μm	0.00	86.96	3.65	<0.0001
2000 μm	7.56	7.71	2.33	0.98
1000 μm	32.36	3.21	2.95	<0.0001
500 μm	28.28	0.86	3.33	<0.0001
250 μm	18.60	0.51	6.04	<0.0001
125 μm	12.82	0.57	4.08	<0.0001
63 μm	0.37	0.16	0.09	<0.0001
45 μm	0.01	0.01	0.002	0.99
23 μm	0.00	0.00	0	0.99
Geometric mean diameter (μm)	717.34	3940.70	4.96	<0.0001
Geometry standard deviation (μm)	2.24	8.53	0.18	<0.0001

[1] Apparent density = volumetric weight (kg/L) was conducted by the ratio of mass of grain to its bulk volume.

[2] Proportion of material remaining above each sieve after 10 min shaking

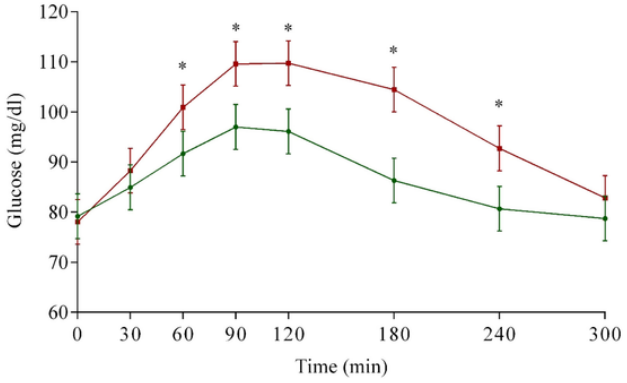

Fig. 2. The effect of processing on the horses' plasma glucose concentrations at different times after feeding (Processing* time: P = 0.06, Processing: $p < 0.001$ and time: $p < 0.001$). The values are least square means and bars show SEM. The asterisks indicate differences between the two treatments at the given time point. For each sampling time and processing method, there were 18 observations (3 starch intake level × 6 replicates).

on plasma glucose concentration (A) and the AUC of plasma glucose responses (C). Micronization increased plasma glucose concentration ($p < 0.001$) from 86.83 to 95.81 mg/dl and a trend was found for an interaction between processing and time (P = 0.06) for this parameter (Fig. 2). The AUC of plasma glucose was greater (P = 0.003) in the horses fed micronized flaked corn (C) than those consuming ground corn (26103 vs 29656 mg/dl/min).

Increasing starch level per meal increased peak plasma glucose concentration between the lowest (1 g/kg BW) and highest (2 g/kg BW) levels (88.60 vs 95.05 mg/dl respectively; P = 0.005; Fig. 3 (B)). No interaction of starch level and time was found for plasma glucose concentration. Increasing starch level per meal increased (P = 0.04) the AUC of plasma glucose response from 26763 to 29495 mg/dl/min in horses that consumed 1 and 2 g/kg BW starch per meal, respectively (Fig 3, D). However, the difference between moderate starch level (1.5 g/kg) with levels 1 and 2 g/kg BW starch per meal was not significant for peak plasma glucose concentration or AUC glucose. There were no interactions of processing and starch level (P = 0.78) and processing, starch level and time (P = 0.97) for plasma glucose concentration.

3.3. *In vitro* DM disappearances

The effect of corn grain processing on the 6 h *in vitro* foregut and 24 h *in vitro* hindgut DM disappearances is displayed in Fig 4. The DM disappearance of ground corn after 6 h *in vitro* enzymatic digestion was lower ($p < 0.001$) than for micronized flaked corn (193.31 vs 594.82 g/kg DM). Hindgut DM disappearance of undigested residues from *in vitro* enzymatic digestion was greater ($p < 0.001$) in the ground corn than in micronized flaked (730.15 vs 317.64 g/kg DM, respectively).

3.4. *In vitro hindgut gas production and final pH*

Cumulative gas production during 24 h *in vitro* hindgut incubation for undigested residues from 6 h *in vitro* enzymatic digestion of ground and micronized flaked corn are shown in Fig. 5 and parameters of gas production kinetics are summarized in Table 2. The potential of gas production (A) was greater in the ground corn residue compared to micronized flaked residue ($p < 0.001$). However, gas production from incubation of undigested micronized flaked corn occurred at a relatively faster rate (K) than indigestible ground corn residues after 6 h enzymatic digestion ($p < 0.001$). There was also a difference between incubated treatments for the parameter 'n' indicating curve shape ($p < 0.001$). No difference between incubated treatments for final culture pH was observed (Table 2).

4. Discussion

Small intestine starch digestibility of corn grain is relatively lower than for oats in horses [3]. Therefore, the main objective of thermal processing for corn grain is to enhance enzymatic starch availability so that less starch enters the hindgut. It is important to measure and report certain indicators of thermal processing success prior to feeding the animal. Flake density [23] and WAI [15] are two indicators for starch gelatinization. Based on the equation developed by Schwandt et al. [23], there is negative correlation between flake density and enzymatic starch accessibility. Greater water absorption capacity also indicates higher starch gelatinization [15]. When starch undergoes gelatinization due to heat treatment, the molecular structure within the starch granule is disrupted, leading to increased starch digestibility and a higher glycemic index when fed to the horses [10].

In the present study, micronized-flaked corn had a lower apparent density and greater WAI compared to ground corn. Pathiratne et al. [24] and Semwal and Meera [25] also reported that infrared heating increased the water holding capacity of the grain. With flake density of 0.32 kg/L, enzymatic starch availability would be calculated as 600 g/kg DM [23]. The observed 6 h DM disappearance in the present study was 594.82 g/kg DM which was very closed to calculated value. These data suggest that the infrared treated corn used in the present study likely was highly gelatinized, although we did not estimate the degree of starch gelatinization.

Enzymatic DM disappearance in the corn grain was increased by about three times as a result of micronization and flaking of corn (flake density of 0.32 kg/L) compared to ground corn. In the present study, the particle size of the grains used *in vitro* were the same size as fed to the animals. In fed conditions, flaked grains are chewed before foregut digestion, thus DM disappearance of the micronized-flaked corn *in vivo* would likely be even more than observed here. There was also a positive correlation between DM and starch disappearance [26]. Therefore, it can be suggested that micronization and flaking enhanced starch digestibility of corn grains used in the current study as expected and in agreement with previous findings [9,21]. Because of the potential improvement in starch digestibility, horses that were fed micronized flaked corn had greater plasma glucose and AUC of plasma glucose compared to animals that consumed ground corn. Our finding was consistent with Hoekstra et al. [27] and Thorringer et al. [9] who observed that feeding steamed- and micronized-flaked corn increased starch digestibility and glucose availability compared to ground corn, although none reported flake density of the processed corn.

Based on the *in vitro* results that with similar enzyme concentration, enzymatic digestion of micronized-flaked corn was three times greater than ground corn, so we expected a similar fold-increase in plasma glucose for horses fed micronized-flaked corn compared to ground corn. This means, AUC would increase from 25475 in horses that were fed ground corn to provide 1 g starch per kg BW/meal to an expected value of 78463 mg/dl/min (25475 × 3.08) in horses that consumed micronized-flaked corn at the same level. However, the observed value for this parameter was 28050 in the horses fed micronized-flaked corn. This shows either starch digestion under *in vivo* conditions was not proportional with the *in vitro* DM or starch disappearances [9] or there was a limitation in small intestine glucose absorption. It should be noted that animals in the current study were fed with corn grain once a day only in sampling days and were not adapted to the corn treatments. Dyer et al. [28] concluded that small intestine capacity for glucose absorption was enhanced by 3.3-fold after proper adaptation which may be why we observed lower-than-expected glucose availability in horses fed micronized-flaked corn compared to the ground corn fed group.

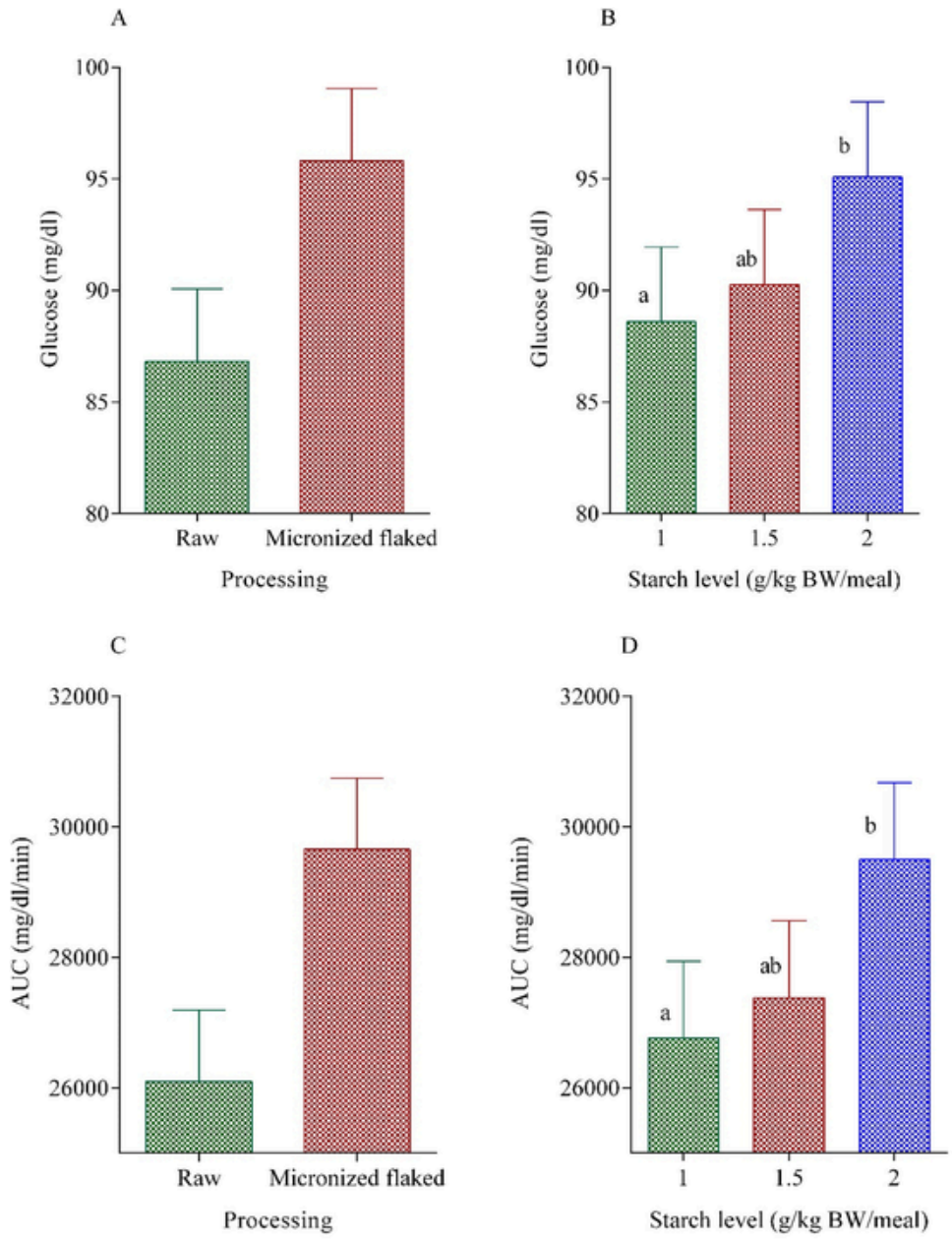

Fig. 3. The effects of corn processing [A: ($p < 0.0001$) and C: (P = 0.0052)] or starch levels per meal [B: (P = 0.0003) and D: (P = 0.037)] on the horses' plasma glucose concentration and area under the curve (AUC) of plasma glucose responses. The values are least square means and bars show SEM. Different letters on the bars represent significant differences. There were 18 replicates for each processing method in the figures A and C (3 starch intake levels × 6 replicates) whereas each starch level in the figures B and D involved 12 replicates (2 processing methods × 6 replicates).

Similarly, with increasing starch level per meal, the enhancement of plasma glucose was not observed in a same manner. For instance, we would expect AUC to double (from 28,050 to 56,100 units) when starch intake doubled from 1 to 2 g/kg BW in horses fed micronized-flaked corn. However, the observed value was 32148 in the horses fed 2 g/kg BW starch per meal through micronized-flaked corn. Our finding is in line with Vervuert et al. [7] who found at starch levels above 1.1 g/kg BW/meal, increases AUC for plasma glucose concentrations were not significant when micronized cereals were fed. In contrast, Gordon et al. [29] found that plasma glucose AUC increased by 2 times when non-fibrous carbohydrate level in the diet was doubled after proper adaptation. Although our finding may have been in part due to the lack of adaptation period to high levels of starch feeding as mentioned earlier, it should be noted that equine pancreatic secretions have low levels of α-amylase activity and the enzymatic hydrolysis of starch is limited [30]. The capacity of glucose absorption in the equine small intestine is also limited [31]. For the above reasons, blood glucose increases did not occur to the same magnitude as increasing starch level per meal.

The above fact again indicates that even when heat-processed grain are fed, increasing starch level per meal may cause undigested starch or unabsorbed glucose to reach the hindgut [9]. In a review study by Julliand et al. [4] on pre-caecal digestibility of various grain starches, maximum starch intake level was determined to be 2 g/kg BW per meal. However, other references established l g/kg BW per meal as a safe level to avoid hindgut acidosis [8]. Overall, feasibility of increasing acceptable starch level per meal by feeding micronized-flaked corn requires more research work in adapted horses.

Studies have shown that the equine hindgut has great potential for fermenting undigested starch reaching the cecum [4]. During the first 14 h of *in vitro* hindgut fermentation, residues of enzymatic digestion

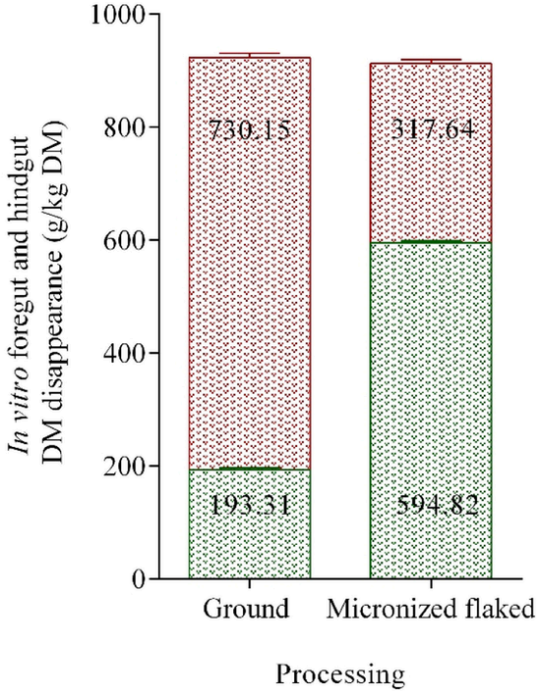

Fig. 4. The effect of processing corn grain on the 6 h *in vitro* foregut ($p < 0.001$) and 24 h *in vitro* hindgut ($p < 0.001$) DM disappearances. The values are least square means and bars show SEM.

were fermented faster in the micronized-flaked than ground corn. Therefore, if highly gelatinized starch reaches this part of the digestive tract, large amounts of acid might be produced within a shorter time compared to the situations that ground corn starch reaches to the hindgut. In our *in vitro* fermentation experiment, the pH was measured at the end point of the batch culture and was similar for both ground and heat-processed corns. However, this single measurement of pH could not capture differences in earlier in the incubation period when rates of degradation were greater for micronized corn. Therefore, continuous monitoring of fecal pH in horses fed increased level of micronized-flaked corn is recommended for future studies to access potential risk of hindgut acidosis when feeding micronized corn.

5. Conclusion

From the results of the present study, it can be concluded that micronization and flaking of corn grain (at flake density of 0.32 kg L) increased enzymatic starch digestibility resulting in higher post prandial blood glucose concentrations. However, increasing starch level in the horses consuming micronized-flaked corn did not enhance plasma glucose as expected. This was potentially due to the lack of dietary starch adaptation, limitations in the enzyme activity, glucose absorption or all of these. Therefore, in addition to feeding processed grains with high digestibility, it is still vital to consider the level of starch consumption in each meal. To increase the efficiency of starch utilization in the small intestine and decrease starch fermentation in the large intestine, micronized flaked corn should be fed at low levels per meal. Repeating this study by providing enough adaptation period is recommended.

Ethics statement

The Ethics Committee of FUM accepted and supervised all of the experimental processes (526/31/10/2021).

Source of research funding

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CRediT authorship contribution statement

Fahimeh Varasteh: Writing – original draft, Investigation, Data curation. **Seyed Hadi Ebrahimi:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Abbas Ali Naserian:** Supervision. **Saeid Zerehdaran:** Supervision, Formal analysis. **Vahideh Heidaraian Miri:** Resources, Methodology, Funding acquisition.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

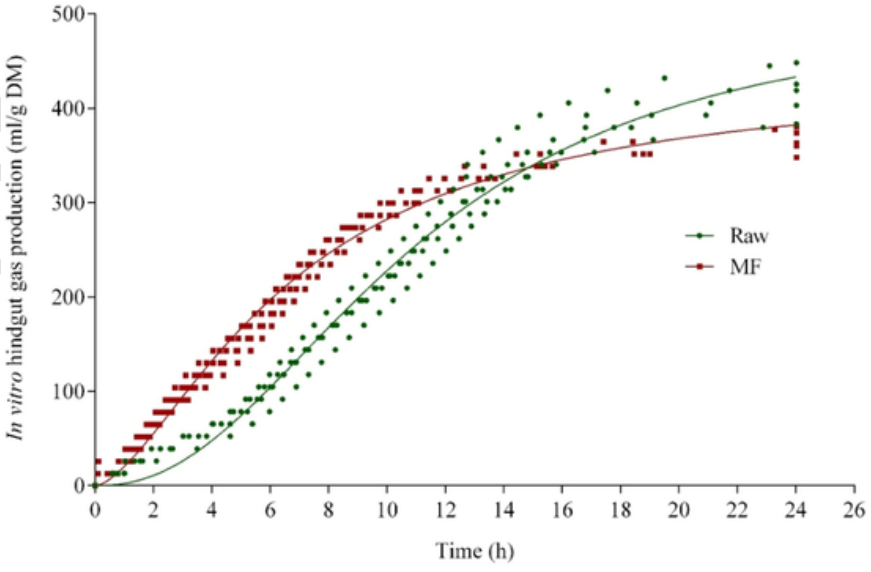

Fig. 5. Cumulative gas production during 24 h *in vitro* hindgut incubation for undigested residues from 6 h *in vitro* enzymatic digestion of ground and micronized flaked corn.

Table 2
The parameters of gas production kinetics and final pH following fermentation of ground and micronized flaked corn grains (The values are least square means).

Parameters[1]	Corn grain processing		SEM	P-value
	Ground	Micronized flaked		
Total gas (ml/g DM)	415.88	364.71	7.08	0.0005
Net gas (ml/g DM)	397.89	346.72	7.08	0.0005
A	507.7	433.5	11.84	0.002
n	2.25	1.58	0.05	<0.0001
K	10.96	6.73	0.29	<0.0001
Final pH	6.43	6.45	0.003	0.18

[1] A is the asymptote gas production (ml/g DM), n is the value determining the shape of the curve and K is the time (h) to produce half of A.

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