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Flaking method affects the rate and extent of ruminal starch degradation in maize grains

Rezvan Samsami, Abbas Ali Naserian, Abdolmansoor Tahmasbi, Seyed Hadi Ebrahimi*

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

*Corresponding author,
E-mail address:
naserian@um.ac.ir

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ORCID
Rezvan Samsami
0000-0001-5389-0658
Abbas Ali Naserian
0000-0003-1179-6262
Abdolmansour Tahmasebi
0000-0001-5979-9783
Seyed Hadi Ebrahimi
0000-0002-0156-0646

Abstract In this study, we investigated the effect of different processing methods (steam-flaking, micronized and micronized-flaking; F, M, and MF, respectively) of corn grains on various factors including water absorption index (WAI), damaged starch content (DSC), nutrient digestibility, gas production (GP), microbial crude protein (MCP) and effective utilizable crude protein (EUCP). Flaked treatments (F and MF) were used in whole, cracked and ground forms (1.5- and 3-mm), while non-processed corn grains (G) and M were only ground. All MF treatments showed higher ruminal digestibility of dry matter (DM) and starch, GP, as well as increased MCP and EUCP compared to other treatments (P<0.001). The F treatment in the whole form and ground micronized corn showed significantly lower (P<0.001) ruminal digestibility of DM and starch, as well as GP and MCP. However, the above two treatments exhibited greater in vitro disappearances of DM and starch in the small intestine. It can be concluded that post-flaking of infrared radiated corn may induce severe ruminal degradation above that of steam flaking. Therefore, a flake density greater than 0.41 kg/L may be appropriate for micronized-flaked corn when used in its whole form to achieve optimum ruminal degradation and maximum digestibility of starch in the small intestine.

Keywords: gas production, starch digestion, flake density, micronization

Introduction

Flaking grains offers two advantages over grinding. First, it uniformly increases the surface area for the action of digestive enzymes. Second, it enhances starch digestibility by applying heat during the process (DePeters et al., 2003). The heat and moisture gelatinize starch molecules, thereby increasing the accessibility of starch polymer to enzymes (Kang et al., 2021). Steam-flaking technology was developed to improve the energetic value of grains, particularly maize grain (Barajas and Zinn, 1998). Additionally, flaked grains can also be produced using infrared radiation technology, which is referred to as micronized-flaked grains (Sajjadi et al., 2022).

Flake density is a well-known characteristic of flaked products. It has been recommended that, for optimal ruminal degradation, the flake density of steam- flaked corn grain should not be below 0.31 kg/L (Zinn et al., 2002). If the flake density is below this value, the rate and extent of ruminal starch degradation may be high, potentially leading to ruminal acidosis (Zinn et al., 2002). However, high flake density, which indicates a high thickness of the flake particles, may result in increased starch escaping from digestion and a reduction in the energetic value of corn grain (Gutierrez et al., 2018). Joy et al. (1997) reported that ruminal starch digestibility decreased from 44.8% to 27.2%, and total-tract digestibility decreased from 94.4% to 85.1%, as flake density increased from 0.31 to 0.39 kg/L. Many studies have been conducted to determine the optimum

flake density of steam-flaked corn grains (Ahmadi et al., 2019). The results indicate that the optimum flake density for corn grain in high-producing dairy cows may be greater than 360 g/L. It should be noted that the above values are documented for steam-flaked corn grains and to our knowledge, an optimum flake density for micronized-flaked corn grain is lacking.

The pre-flaking process involves heating grains using either steam in a steam chest or infrared radiation on a monolayer of substances as they move beneath the radiation source. When using steam, the temperature in the steam chest is approximately 95°C at atmospheric pressure, and corn grains require a retention time of 30 minutes (Dehghan-banadaky et al., 2007). To ensure uniform heating of all seeds, an adequate number of steam nozzles must be distributed throughout the steam chest. In the micronization process, the grains are heated to achieve a surface temperature of 135°C before they are flaked (Serna-Saldivar, 2016). For uniform heating through infrared radiation, the grains must vibrate as they move under the radiation source (Sajjadi et al., 2022). It is assumed that all the seeds move at a similar speed beneath the radiation source. However, the vibration of the conveyor can cause random movement of grains with different weights, resulting in varying retention times during heating. Consequently, some grains may become overheated.

We hypothesized that micronized-flaked and steam-flaked corn grains with the same flaking condition post-heating, would have similar flake density, extent and rate of ruminal starch degradation. Therefore, the objective of the present study was to investigate the above hypothesis using in situ and in vitro experiments.

Materials and methods

Sample preparation

A batch of corn grain (Single Crass 704) was obtained from the Orzuiyeh agricultural farm in Kerman, Iran. The treatments included steam-flaked (F), micronized (M), micronized-flaked (MF) and non-processed corn grains (G). In the ground corn treatment (G), corn grains were finely ground using a hammer mill (model MS90L 1-2, KW 2.2, R.P.M 2840, class F, Mashhad, Iran) equipped with a 1.5- and 3-mm screen (G1.5m and G3m, respectively). For the steam-flaking process, the whole corn grains were steamed in a vertical steam chamber for approximately 30 minutes at 95°C to increase the moisture level to 180-200 g/kg. The steamed grains were then flaked using a flaker machine with two rollers set at a 1-mm gap, resulting in a density of about 450 g/L. The steam-flaked corn grains (F) were used as whole flakes (WF), cracked into four pieces (CF) or ground to pass through 1.5 and 3-mm sieve holes (F1.5m and F3m, respectively). To produce micronized corn (M), the grains were soaked in tap water and kept at room temperature (25±2°C) for 6 hours to achieve an initial moisture content of 150-180 g/kg. The grains were then exposed to infrared rays in a commercial micronizer (Faravardaneh Ferdowsi Mashhad, Ltd. Mashhad, Iran) equipped with natural gas-fired ceramic burners. The surface temperature of the seeds reached 135°C as they exited the micronizer. The heated grains were cooled and ground using a hammer mill (model MS90L 1-2, KW 2.2, R.P.M 2840, class F, Mashhad, Iran) with a 1.5 or 3-mm screen (M1.5m and M3m, respectively). For the micronized-flaking process, the micronized grains were immediately flaked using a flaker machine similar to the one used for producing steam-flaked corn grains, resulting in a density of about 410 g/L. This treatment (MF) was used in various forms: whole (WMF), cracked into four pieces (CMF) and ground to pass through 1.5 and 3-mm sieve holes (MF1.5m and MF3m, respectively).

Chemical analysis

Samples of grains were analyzed in triplicate to determine their dry matter (DM), ash, crude protein (CP), and ether extracts (EE) content using the AOAC (2012) procedure. The DM content was determined by oven drying a subsample at 100°C (934.01). Ash and EE contents were analyzed using methods 945.38 and 945.18, respectively. Total nitrogen (N) was determined using the Kjeldahl method (997.06) with a Kjel-Foss, Kjeltec Auto 1030, and CP was calculated as N multiplied by 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed based on the method described by Van Soest et al. (1991). The total starch content was determined using the method outlined by Rose et al. (1991). Non-fibrous carbohydrate (NFC) and metabolizable energy (ME) were calculated using the equations proposed by Sniffen et al. (1992) and Menke and Steingass (1988):

NFC (%) =100 - (CP + NDF + EE+ ash)

Where: NFC= non-fibrous carbohydrate, CP= crude protein, NDF= neutral detergent fiber and EE= ether extract (percent of DM basis).

ME (MJ/kg DM) = 0.157×GP+0.0084×CP+0.022×EE-0.0081×CA+1.06

Where:

ME= metabolizable energy, GP= 24-h net gas production (mL/200 mg DM), CP= crude protein, EE= ether extract and CA= crude ash (percent of DM basis).

Physical properties

The densities of micronized flaked and ground samples were measured using the procedure described by Schwandt et al. (2017). This involved determining the ratio of the grain's mass to bulk volume. The particle size distribution of the samples was measured in triplicate by dry sieving a representative 100 g sample through sieves of 4000, 2000, 1000, 500, 250, 125, 63 and 45 µm for 10 minutes. A vibratory sieve shaker (Restch AS 200, Germany) was used for this process. The amount of material retained on each screen size was then

determined. Additionally, the geometric mean diameter (GMD) and geometric standard deviation (GSD) of the sample were calculated as described by Amerah et al. (2007).

In situ and in vitro dry matter, starch and protein disappearances

Ruminal-intestinal DM, starch and CP disappearance were determined using the *in situ* and *in vitro* methods (Ngonyamo-Majee et al., 2009), as previously described by Calsamiglia and Stern (1995). Two lactating Holstein cows, fitted with ruminal cannulas, were fed a diet consisting of 700 g/kg hay and 300 g/kg concentrate, equivalent to 3.5% of their body weight. The concentrate included 450 g/kg barley, 400 g/kg beet pulp, 100 g/kg soybean, 30 g/kg beet molasses, and 20 g/kg mineral-vitamin premix. The daily ration was divided into two equal portions and fed at 08:00 and 17:00, respectively. Approximately 7 g of the samples were placed into nylon bags (9 × 17 cm) with a pore size of 50 µm. The bags were inserted into the rumen just before the morning meal and left there for 12 hours with four replicates. After removal from the rumen, the bags were rinsed with cold water and then washed three times in a washing machine, with each wash lasting 2 minutes. Subsequently, the bags were dried at 60°C for 48 hours to determine the DM content. Zero-hour bags were also immersed in water at a temperature of 39°C for 15 minutes before washing to evaluate the soluble DM or fine particles that escape through the bag pores.

Samples of residue from the bags (residue containing 15 mg of N) were subjected to enzymatic incubation to simulate post-ruminal digestion. First, the samples were incubated in pepsin for 2 hours. Then, they were rinsed and incubated in pancreatin buffer for 6 hours. Afterward, the residue was washed and dried in an oven at 60°C for 48 hours. The DM content of the residue was measured to calculate DM disappearance. The starch content of the residue was determined using the method described by Rose et al. (1991), and subsequently, starch disappearance was calculated. The CP content of the residue was determined using AOAC (2012) analysis, and then the protein disappearance was calculated.

In vitro rumen fermentation and microbial protein yield

The rumen fermentation and MCP were determined using the *in vitro* GP technique (Grings et al., 2005), which was previously described by Blummel and Lebzien (2001). Substrates (250 mg) were added to 100 mL serum bottles in triplicate for each treatment group. Rumen fluid was collected from two non-pregnant lactating Holstein cows (650 kg body weight), with a permanent fistula, before the morning feeding. These cows were fed a diet consisting forage and concentrate in a 50:50 ratio twice a day (at 8:00 and 16:00) to meet the nutrient requirements for 30 kg of milk production (NRC, 2000). After collection, the rumen fluid mixture was placed into pre-warmed thermo flasks to transport to the laboratory within half an hour, and filtered through four layers of sterilized cheesecloth to remove any solid particles.

Twenty milliliters of buffered rumen fluid medium were added to serum bottles under anaerobic conditions. The bottles were tightly sealed with a rubber stopper and aluminum cap, and then placed in a water bath at a temperature of 39°C for 96 hours. Blank samples, consisting only of the medium without any substrate, were also placed in the water bath to measure the GP solely from the medium itself, eliminating any potential influence from a substrate. The gas volumes were determined by measuring the headspace pressure at various time points (2, 4, 6, 8, 10, 12, 14, 16, 24, 30, 36, 48, 54, 60, 72, and 96 hours) during the incubation period. After correcting for the GP of the blanks, the GP data were fitted to the generalized Michaelis-Menten model without a lag phase (France et al., 2000) as follows:

$$GP = A \times (T^n/(T^n + k^n))$$

Where:

GP= the gas production at time T, A= the asymptote GP (mL), n= determines the shape of the curve, k= the time to produce half of A. The other parameters were calculated according to Groot et al. (1996) and France et al. (2000):

Degradation rate at half-life (c)= n/(2×k)

The second incubation was performed to determine the rumen MCP yield at specific times ($t_{1/2}$) for each treatment. The $t_{1/2}$ is equal to the time (hour) when half of the asymptotic gas volume (mL) has been produced.

The microbial nitrogen production at $t_{1/2}$ was estimated by using the following equation:

Microbial nitrogen production at $t_{1/2}$= Diet N + (ΔNH_3-N) - NDFN at $t_{1/2}$

ΔNH_3-N= NH_3-N in blanks (0h) − NH_3-N in diet incubations at $t_{1/2}$

Where:

Diet N= nitrogen content of the experimental diets, NH_3-N in blanks= average amount (mg) of NH_3-N in the blanks before incubation (0.0 h), NH_3-N in diet incubations at $t_{1/2}$= amount (mg) of NH_3-N in the incubation bottles from each sample at $t_{1/2}$, NDFN at $t_{1/2}$= nitrogen content of the truly non-degraded residue at $t_{1/2}$. Microbial nitrogen production at $t_{1/2}$ is converted to MCP using a coefficient of 6.25.

Effective utilizable crude protein in the duodenum

The effective UCP in the duodenum was determined using the technique developed by Edmunds et al. (2012). In this method, the modified Hohenheim GP technique (modHGT), as described by Steingass et al. (2001), was used to measure NH_3-N after incubation. Three runs with four replicates for each incubation time were carried out. The modHGT follows the HGT procedure (Menke and Steingass, 1988) with a specific chemical modification: an increase of 2 g/L in NH_4HCO_3

and a decrease of 2 g/L in $NaHCO_3$ in the buffer solution. Sampling was performed at 8, 24, and 48 hours of the incubation. After opening the bottle caps, 5 mL of the liquid phase was pipetted into 50 mL serum bottles containing 5 mL of 0.2 N HCl. The bottles were carefully sealed with a rubber stopper and aluminum caps, then stored at a temperature of 4°C for measuring the concentration of NH_3-N using the method described by Weatherburn (1967). The value of UCP was estimated using the following equation:

UCP (g/kg DM) = [(blank NH_3-N + sample N − liquid NH_3-N) × 6.25 × 1000]/S

Where:

UCP= utilizable crude protein, blank NH_3-N= the amount of NH_3-N in the blanks after incubation (mg), sample N= the nitrogen amount of the incubated sample (mg), liquid NH_3-N= the amount of NH_3-N in the liquid contained in the syringes after incubation (mg), S= the total amount of sample on DM basis (mg).

The UCP values from two different incubation time points in one run were plotted against a log time (ln t), where 't' represents the time of incubation. The created regression equation was used to estimate the effective utilizable crude protein (EUCP) using the following formula:

$$\mathrm{EUCP} = \mathrm{b} + \mathrm{a} \times \mathrm{In}\,(\frac{1}{Kp})$$

Where:

EUCP= Effective utilizable crude protein, b= the intercept of the regression equation, a= slope of the regression equation, k_p= assumed fractional passage rate at 0.02, 0.04, 0.06 and 0.08/ h.

Starch damage

The DSC was determined using the amperometric method (Chopin, ZI Val de Sein, 92390 VLG, France). This method is based the research conducted by Medcalf and Gilles (1965), who found that the iodine absorption capacity of grain flour is directly proportional to the starch damage content

Water absorption index

The WAI of the samples was measured using the method described by Zarzycki et al. (2017). It was calculated using the following formula:

WAI (%) = (W_g/WDM) × 100

Where:

WAI= water absorption index, W_g= weight of DM of sample plus absorbed water, WDM= DM of original sample before soaking.

Statistical analysis

All data were analyzed as a completely randomized design using the general linear model (GLM) procedure of Minitab software (Minitab 16) with flowing statistically model:

$$y=\mu+T_i+ e_{ij}$$

Where: y= dependent variable, μ= overall mean, T_i= effect of treatment, e_{ij}= residual error

Orthogonal contrasts were constructed to evaluate the effect of corn processing (flaking, micronization and micronized-flaking) versus untreated (G) for chemical analysis and *in vitro* measurements.

Results

Chemical compositions

The chemical composition, ME, DSC and WAI of corn grains are presented in Table 1. The difference in DM content between G and MF was not significant but they had lower and higher DM content than F and M, respectively (P<0.01). Heat processing significantly increased NDF, ADF and starch content in corn grain compared to G treatment (P<0.001). There were no significant differences in organic matter (OM), CP, Ash and EE contents of the experimental treatments. Flaking after micronization or steaming increased (P<0.001) WAI, DSC and ME, with the highest values observed in MF. However, in M treatment, the ME was reduced (P<0.001) despite the increases in WAI and DSC.

Physical properties

The apparent density and geometric mean particle size of the processed corns are shown in Table 2. The density of WF (0.45 kg/L) and WMF (0.41 kg/L) was similar and increased with the reduction of particle size. The greater mean particle size (μm) was observed in unground treatments as follow: WMF (4225), WF (3321), CMF (2514) and CF (1940) compared to ground treatments with 3mm and 1.5mm screen (423 to 962). We found that 61.55% of the WF and 93.12% of the WMF particles were retained on a 4 mm sieve. Additionally, the number of particles that passed through screens with a mesh size less than 1mm increased with grinding (from 3mm to 1.5mm screens).

In situ and in vitro dry matter, starch and protein disappearances

Table 3 presents the *in situ* and *in vitro* DM, starch and CP disappearances for different types of processed corn grains. The MF3m showed the highest ruminal DM disappearance (P<0.001), followed by CMF or WMF with no significant difference between the last two. In relation to F treatments, WF had the lowest ruminal DM disappearance but this parameter was increased by physically reducing the particle size (P<0.001). Despite having a similar particle size, F3m had a higher ruminal DM disappearance than the control. Surprisingly, M3m had significantly lower ruminal DM disappearance than the control.

Table 1. Chemical composition (%DM basis) and physical properties of processed corn grain

Item	Processed corn grain [1]					
	G	F	M	MF	SEM	P-value
Chemical composition (%) [2]						
DM	91.28[b]	92.31[a]	90.77[c]	91.68[b]	0.110	<0.001
OM	98.91	98.91	98.90	98.91	0.002	0.09
CP	9.66	9.66	9.66	9.65	0.010	0.87
NDF	10.29[d]	11.44[c]	14.20[a]	12.51[b]	0.150	<0.001
ADF	2.31[c]	2.55[a]	2.44[b]	2.41[b]	0.020	<0.001
Starch	69.44[c]	73.17[b]	73.61[b]	74.60[a]	0.110	<0.001
Ash	1.09	1.09	1.10	1.10	0.002	0.10
Ether extract	5.04	4.98	5.02	4.99	0.020	0.12
Non-fibrous carbohydrate [3]	73.91[a]	72.83[b]	70.03[d]	71.75[c]	0.140	<0.001
Metabolizable energy [4] (MJ/kg DM)	13.61[c]	13.73[b]	13.42[d]	13.84[a]	0.020	<0.001
Damaged starch content [5] (g/kg)	19.03[c]	32.80[b]	33.40[b]	35.10[a]	0.210	<0.001
Water absorption index [6]	259[d]	285[c]	337[b]	353[a]	0.640	<0.001

[1] Processed corn grains were ground corn (G), steam-flaked corn (F), micronized corn (M) and micronized-flaked corn (MF).
[2] Dry matter (DM); organic matter (OM); crude protein (CP); Neutral detergent fiber (NDF), Acid detergent fiber (ADF); starch; ash; Ether extract (EE).
[3] Estimated according to Sniffen et al. (1992), non-fibrous carbohydrate (NFC) =100 - (CP + NDF + fat+ ash)
[4] Estimated according to Menke and Steingass (1988), metabolizable energy (MJ/kg DM) = $0.157 \times \mathrm{GP} + 0.0084 \times \mathrm{CP} + 0.022 \times \mathrm{EE} - 0.0081 \times \mathrm{CA} + 1.06$
[5] Damaged starch content (DSC)- determined using the method of Medcalf and Gilles (1965).
[6] The water absorption index (WAI) determined by the method of Zarzycki et al. (2017)
SEM= Standard error of the mean
a,b: Within rows, means with common superscript (s) are not different (P>0.05)

Table 2. Distribution of processed corn grain particles on the sieves (%)

Item	Processed corn grain[1]											
	G3m	G1.5m	WF	CF	F3m	F1.5m	M3m	M1.5m	WMF	CMF	MF3m	MF1.5m
Apparent density (Kg/L)[2]	0.66 ± 0.05	0.72 ± 0.08	0.45 ± 0.065	0.63 ± 0.04	0.68 ± 0.02	0.73 ± 0.07	0.65 ± 0.03	0.7 ± 0.01	0.41 ± 0.05	0.53 ± 0.02	0.69 ± 0.06	0.74 ± 0.03
Sieve opening, (mm)												
4	0.00	0.00	61.55	18.32	0.00	0.00	0.00	0.00	93.12	32.71	0.00	0.00
2	3.00	0.14	28.23	41.02	0.61	0.10	14.14	0.14	5.26	42.65	0.39	0.13
1	35.51	3.67	6.41	25.23	25.53	6.86	44.89	5.15	0.65	15.68	23.29	2.77
0.5	48.36	52.86	1.57	7.92	38.43	42.02	18.48	52.58	0.32	5.22	37.54	43.72
0.25	6.50	36.80	1.05	4.67	33.00	34.59	16.19	35.43	0.23	2.47	31.05	30.62
0.125	6.25	6.35	1.12	2.77	2.27	16.44	6.26	6.49	0.38	1.22	7.63	21.72
0.063	0.38	0.18	0.06	0.08	0.16	0.00	0.03	0.21	0.03	0.04	0.11	1.04
0.045	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GMPS [3] (µm)	820	514	3321	1940	654	465	962	523	4225	2514	605	423
STD [4] (µm)	1.84	1.61	1.74	2.16	1.79	1.79	2.16	1.63	1.34	1.94	1.88	1.82

[1]Processed corn grains were corn ground with a 3 mm screen (G3m), corn ground with a 1.5 mm screen (G1.5m), whole steam-flaked corn (WF), cracked steam-flaked corn (CF), steam-flaked corn ground with a 3 mm screen (F3m), steam-flaked corn ground with a 1.5 mm screen (F1.5m), micronized corn ground with a 3 mm screen (M3m), micronized corn ground with a 1.5 mm screen (M1.5m), whole micronized-flaked corn (WMF), cracked micronized-flaked corn (CMF), micronized-flaked corn ground with a 3 mm screen (MF3m) and micronized-flaked corn ground with a 1.5 mm screen (MF1.5m).
[2] Apparent density (kg/L) was determined using the procedure described by Schwandt et al. (2017) by weighing 1 liter of the processed sample.
[3,4] The geometric mean particle size (GMPS) and standard deviation (STD) were determined using the Amerah et al. (2007) method.

Although, MF treatments (WMF, CMF and MF3m) had greater (P<0.001) ruminal starch disappearance than F treatments (WF, CF and F3m) and reducing particle size by breaking the flakes or grinding enhanced this parameter in both types of heat processed treatments. All of the heat processed corn grains showed lower ruminal CP disappearance than unprocessed corn (P<0.001). The WF treatment had the highest *in vitro* small intestine disappearance of DM (IVSIDMD) at 40.27 %, followed by the M3m treatment at 36.2 %. The MF treatments had the lowest IVSIDMD (P<0.001). Similarly, the WF had the highest *in vitro* small intestine starch disappearance, while the MF3m had the lowest. Unlike ruminal CP disappearance, the G3m treatment had the lowest *in vitro* small intestine CP disappearance compared to the other treatments (P<0.001).

Parameters of in vitro gas production kinetics

Cumulative gas volume and parameters of *in vitro* GP kinetics obtained by fitting gas data with non-linear model are summarized in Table 4. The MF treatments showed maximum GP, asymptote GP (A) and

degradation rate at half-life (c) than other treatments (P<0.001). However, the value of n (which determines the shape of the curve) and the time required for the production of half of A (k) were the lowest (P<0.001). The F treatments had a higher GP and kinetics parameters - (A, c) than the G with a similar particle size (P<0.001). Additionally, WF had the lowest GP, A and c than other treatments. The kinetic parameter (k) was also lower in F treatments compared to the control with a similar particle size. However, WF represented the highest value for the same parameter (P<0.001).

Table 3. The DM, CP and starch disappearances of the corn grain processed by different methods

Item	Processed corn grain[1]								SEM	P-value
	G3m	WF	CF	F3m	M3m	WMF	CMF	MF3m		
Rumen disappearance (%)										
Dry matter	54.61^{d}	40.50^{g}	50.98^{e}	56.83^{c}	44.44^{f}	61.17^{b}	62.18^{b}	65.07^{a}	0.61	<0.001
Crude protein	53.28^{a}	26.71^{ed}	41.89^{b}	43.84^{b}	34.56^{c}	24.19^{e}	28.25^{d}	44.89^{b}	1.11	<0.001
Starch	64.28^{de}	57.83^{f}	63.18^{e}	66.98^{d}	62.22^{e}	70.27^{c}	73.41^{b}	81.40^{a}	0.68	<0.001
Small intestine disappearance (%)										
Dry matter	25.30^{d}	40.27^{a}	28.73^{c}	24.39^{d}	36.20^{b}	21.20^{e}	21.10^{e}	20.38^{e}	0.33	<0.001
Crude protein	33.75^{d}	55.57^{ab}	43.80^{c}	43.75^{c}	44.93^{c}	57.62^{a}	53.43^{b}	42.08^{c}	0.97	<0.001
Starch	24.50^{d}	33.59^{a}	26.55^{c}	23.13^{d}	31.74^{b}	21.19^{e}	20.51^{e}	16.14^{f}	0.55	<0.001

[1]Processed corn grains were corn ground with a 3 mm screen (G3m), whole steam-flaked corn (WF), cracked steam-flaked corn (CF), steam-flaked corn ground with a 3 mm screen (F3m), micronized corn ground with a 3 mm screen (M3m), whole micronized-flaked corn (WMF), cracked micronized-flaked corn (CMF) and micronized-flaked corn ground with a 3 mm screen (MF3m)
SEM= Standard error of the mean
a,b: Within rows, means with common superscript (s) are not different (P>0.05)

Table 4. *In vitro* gas production parameters of processed corn grain

Item	Processed corn grain[1]												SEM	P-Value
	G3m	G1.5m	WF	CF	F3m	F1.5m	M3m	M1.5m	WMF	CMF	MF3m	MF1.5m		
Gas production parameters[2]														
A (mL)	582^{g}	593^{f}	493^{j}	574^{h}	583^{g}	604^{e}	557^{i}	572^{h}	617^{d}	621^{c}	626^{b}	629^{a}	0.93	<0.001
n	1.28^{d}	1.23^{f}	1.35^{a}	1.31^{c}	1.25^{e}	1.23^{f}	1.33^{b}	1.32^{bc}	1.21^{g}	1.19^{h}	1.17^{i}	1.17^{i}	0.003	<0.001
k (h)	20.57^{d}	16.76^{f}	34.50^{a}	20.75^{d}	18.12^{e}	16.12^{g}	30.50^{b}	25.12^{c}	15.50^{h}	12.97^{i}	9.35^{j}	8.33^{k}	0.13	<0.001
c (/h)	0.030^{g}	0.036^{e}	0.020^{j}	0.030^{g}	0.034^{f}	0.040^{d}	0.022^{i}	0.026^{h}	0.040^{d}	0.050^{c}	0.060^{b}	0.070^{a}	0.0003	<0.001
Gas production[3](mL/g OM)														
GP (6 h)	93.11^{g}	120.10^{e}	65.82^{k}	83.41^{h}	117.13^{f}	121.54^{e}	69.40^{j}	80.10^{i}	123.71^{d}	139.65^{c}	175.2^{b}	225.5^{a}	0.60	<0.001
GP (12 h)	194.4^{g}	224.3^{e}	162.9^{k}	186.8^{h}	210.4^{f}	225.3^{e}	179.4^{j}	181.2^{i}	235.3^{d}	243.4^{c}	283.6^{b}	323.3^{a}	0.55	<0.001
GP (24 h)	307.3^{h}	334.2^{f}	243.4^{l}	303.2^{i}	315.4^{g}	337.2^{e}	257.3^{k}	284.9^{j}	342.5^{d}	351.0^{c}	362.3^{b}	403.7^{a}	0.66	<0.001
GP (48 h)	395.8^{h}	405.2^{f}	342.3^{l}	371.1^{j}	402.6^{g}	410.2^{e}	361.2^{k}	365.5^{j}	420.2^{d}	429.3^{c}	434.0^{b}	455.6^{a}	0.33	<0.001

[1]Processed corn grains were corn ground with a 3 mm screen (G3m), corn ground with a 1.5 mm screen (G1.5m), whole steam-flaked corn (WF), cracked steam-flaked corn (CF), steam-flaked corn ground with a 3 mm screen (F3m), steam-flaked corn ground with a 1.5 mm screen (F1.5m), micronized corn ground with a 3 mm screen (M3m), micronized corn ground with a 1.5 mm screen (M1.5m), whole micronized-flaked corn (WMF), cracked micronized-flaked corn (CMF), micronized-flaked corn ground with a 3 mm screen (MF3m) and micronized-flaked corn ground with a 1.5 mm screen (MF1.5m).
[2]A= asymptote gas production, n= value determining the shape of the curve, k= time to produce half of A, c= the degradation rate at half-life (/h), which can be calculated using the formula: c= n/(2×k)
[3]GP= Gas production, which can be calculated using the formula: $GP= A\times(T^{n}/(T^{n}\times K^{n}))$
SEM= Standard error of the mean
a,b: Within rows, means with common superscript (s) are not different (P>0.05)

The M treatments with similar particle size had lower GP and kinetics parameters (A, c) compared to control. However, these treatments had greater (n) and (k) despite both having a similar particle size **(**P<0.001**)**. Overall, in all treatments, the kinetic parameter (k) of GP decreased when the particle size was decreased, and vice versa.

In vitro microbial protein yields and effective utilizable CP in the duodenum

The true substrate degradability and nitrogen metabolization, including MCP and EUCP, are presented in Table 5. The MF treatments showed higher MCP compared to the other treatments (P<0.001). Despite having a similar particle size, the F treatments had higher MCP than G, while WF had the lowest value among all the treatments (P<0.001), followed by the M treatments (P<0.001). At any ruminal outflow rate, MF treatments had the highest EUCP (P<0.001). F and M treatments had higher EUCP than the G with a similar particle size (P<0.001)**,** although WF had the lowest value among all the treatments (P<0.001).

Discussion

As mentioned in the materials and methods section, water was added to the corn grains before heat treatments. However, after processing, which involved drying and cooling post-flaking, the final DM content of the products was reduced below that of untreated corn grains. Arntfield et al. (2001) also reported a reduced DM content in seeds after infrared heating. Only the M treatment had the lowest DM probably because those

grains were not flaked, which failed to remove moisture from the grain. However, other studies found that DM content was increased by micronization compared to raw grains in the absence of flaking (Zheng et al., 1998; Mustafa et al., 2002; 2003). Increased fiber fractions observed in the heat-treated grains were also found in previous studies as reported by Savari et al. (2018) and Mustafa et al. (2002). However other authors reported, reduced fiber content in the heat-treated grains (Douglas et al., 1991; Zilic et al., 2010).

Table 5. The MCP, *in vitro* DM disappearance at $t_{1/2}$ and EUCP presented at assumed passage rates of 0.02, 0.04, 0.06 and 0.08/h of treatments

Item	Processed corn grain[1]													
	G3m	G1.5m	WF	CF	F3m	F1.5m	M3m	M1.5m	WMF	CMF	MF3m	MF1.5m	SEM	P-Value
Microbial crude protein (g CP/100g OMD)	4.82^{g}	5.74^{f}	0.24^{j}	4.28^{h}	5.51^{f}	6.66^{e}	3.12^{i}	3.88^{h}	7.35^{d}	10.34^{c}	12.33^{b}	13.00^{a}	0.19	<0.001
Dry matter disappearance (g/kg DM)	544.77^{g}	571.06^{e}	520.89^{j}	533.60^{hi}	562.09^{f}	572.74^{e}	532.09^{j}	536.40^{h}	579.46^{d}	583.82^{c}	591.21^{b}	598.70^{a}	1.2	<0.001
Effective utilizable crude protein (g/kg DM)														
r=0.02/h	82.03^{k}	84.00^{j}	68.85^{l}	93.98^{g}	96.88^{f}	97.82^{e}	90.23^{i}	90.88^{h}	128.96^{d}	156.02^{c}	157.90^{b}	159.00^{a}	0.14	<0.001
r=0.04/h	83.98^{k}	85.85^{j}	70.88^{l}	95.85^{g}	98.75^{f}	99.81^{e}	91.86^{i}	92.92^{h}	130.62^{d}	157.67^{c}	159.62^{b}	160.84^{a}	0.09	<0.001
r=0.06/h	84.90^{i}	87.12^{h}	72.08^{j}	93.12^{g}	100.00^{e}	101.11^{d}	93.16^{g}	93.87^{f}	132.05^{c}	159.13^{b}	160.85^{a}	160.96^{a}	0.11	<0.001
r=0.08/h	86.18^{h}	89.06^{g}	73.74^{i}	94.93^{f}	101.91^{e}	102.97^{d}	94.95^{f}	94.96^{f}	134.04^{c}	161.04^{b}	162.77^{a}	162.85^{a}	0.14	<0.001

[1]Processed corn grains contained corn ground with a 3 mm screen (G3m), corn ground with a 1.5 mm screen (G1.5m), whole steam-flaked corn (WF), cracked steam-flaked corn (CF), steam-flaked corn ground with a 3 mm screen (F3m), steam-flaked corn ground with a 1.5 mm screen (F1.5m), micronized corn ground with a 3 mm screen (M3m), micronized corn ground with a 1.5 mm screen (M1.5m), whole micronized-flaked corn (WMF), cracked micronized-flaked corn (CMF), micronized-flaked corn ground with a 3 mm screen (MF3m) and micronized-flaked corn ground with a 1.5 mm screen (MF1.5m).

SEM= Standard error of the mean

a,b: Within rows, means with common superscript (s) are not different (P>0.05)

Contradictory results were reported for starch content of the heat-treated grains as the starch content was increased (DePeters et al., 2003; Zarkadas and Wiseman, 2002), decreased (Qiao et al., 2015; McAllister and Sultana, 2011) or not changed post-heat treatment (Malcolm and Kiesling, 1993; Fasina et al., 1999). Water and heat disrupt the intra molecular bonds, enhancing the use of water in the hydrogen bonding sites in starch, resulting in increased swelling (Svihus et al., 2005). Therefore, as expected, all the treatment groups in which corn grains underwent a heat process by steam or infrared radiation had a relatively greater WAI than G. In line with the WAI, the DSC increased in the heat-processed grains, indicating greater starch gelatinization as previously reported (Morrison et al., 1994; Vallons and Arendt, 2009). The ME was calculated using an *in vitro* gas dependent formula. The lower ME for M than the other treatments may be a result of lower extent of GP at 24 hours (Paya et al., 2007).

The density and particle size are affected by different processing methods (Yu et al., 1998). Zinn et al. (2002) stated that the density of flaked-corn grain ranges from 0.31 kg/L to 0.41 kg/ L. Ahmadi et al. (2019) reported that the optimum density for flaked corn grains is greater than 360 g/L for high-yielding dairy cows. The density increases as the particle size decreases, which can be related to the decrease in volume and the number of empty internal spaces that can be filled with gas (Giger-Reverdin et al., 2000). In the present study, flake densities were found to be 0.41 and 0.45 kg/L in WMF and WF, respectively. The higher flake density observed in WF compared to WMF may be a result of a lower mean particle size (Bernhart and Fasina, 2009), despite using the same roller distances in both flaking methods (1 mm). The differences in particle size could potentially be attributed to variations in kernel hardness (Murthy et al., 2008) which indicates greater stability in WMF after production (during transport and storage).

As expected, flaked treatments (WF and WMF) had a greater mean particle size than ground corns. The higher proportion of particles on the 4 mm screen due to flaking compared to other treatments is similar to the results of previous studies (Drouillard and Reinhardt, 2007). Based on these observations, Rafiee-Yarandi et al. (2019a) and Savari et al. (2018) found that diets containing steam-flaked corn showed a higher proportion of particles retained on an 8-mm sieve and greater MPS compared to control diets. The increase in the number of particles passed through screens with a mesh size <1 mm by reducing the size of particles was similar to the results of previous studies (Yu et al., 1998), where 54% of finely ground corn and 20% of coarsely ground corn passed through screens with a mesh size <1 mm.

The main factor that affects the extent and rate of ruminal starch degradation in ground corn grain is the mean particle size. Reducing the mean particle size from 3.7 to 0.7 mm increased the ruminal starch degradation of corn grain from 35.5% to 58.6%, respectively (Remond et al., 2004). In the case of flaked corn grains, the degree of starch gelatinization and flake density play significant roles in ruminal starch degradation (Trotta et al., 2021). Although, starch gelatinization was not measured in the present study, it was previously shown that MF treatment had higher WAI and DSC compared to G and F treatments. These two factors are directly related to starch gelatinization (Morrison et al., 1994; Lazou and Krokida, 2010). Therefore, it can be expected that ruminal DM and starch disappearances would be greater in MF treatments compared to F or G treatments with similar particle sizes.

Sajjadi et al. (2022) found that when the degree of starch gelatinization exceeded the degree of protein

denaturation during heat processing, the protein barrier failed to protect the starch gelatinization. This explains why a majority of the starch in MF treatments, which underwent extensive starch gelatinization degraded in the rumen. It has been observed that even with intensive gelatinization, the ruminal starch degradability of corn grain can remain low if the particle size is not sufficiently reduced (Kang et al., 2021). In this study, the ruminal starch disappearance was higher in WMF compared to WF, despite the larger mean particle size and relatively lower flake density of WMF. Therefore, contrary to the findings of Kang et al. (2021), starch gelatinization is dominated to particle size for effecting on ruminal starch degradation.

The results of the present study also show an exception, which is M treatment. This treatment involved grinding micronized whole corn grain after it had been cooled and stored. This different processing and preparation method resulted in a completely different ruminal behavior compared to MF treatments even with similar particle size. According to McAllister and Sultana (2011) and Semwal and Meera (2020), it is hypothesized that infrared radiation creates intermolecular disulfide-cross linkages between sulfur-containing amino acids and increases the disulfide-bonded protein matrix surrounding starch granules. This matrix may be further disrupted by flaking or fine grinding. We speculate that this protective layer forms more strongly in grains that have been micronized but not immediately flaked and have been stored for a long period after cooling.

One of the main objectives of processing is to shift the site of digestion from the rumen to the small intestine (Owens et al., 1986). In general, it is desirable to achieve the highest enzymatic digestion in the small intestine for both starch and protein (Arieli et al., 2001; Mustafa et al., 2000). Surprisingly, WF followed by M3m exhibited greater intestinal disappearance of DM and starch than other treatments. It is likely that in the first step of the *in vitro* experiment, the physical form and protein matrix surrounding the starch granules protected the starch from ruminal degradation. However, in the second step (gastric acidic conditions), the protein barrier was disrupted and the gelatinized starch underwent greater enzymatic digestion in the small intestine.

As a general fact, increasing ruminal degradability in energy concentrate substances by reducing particle size, enhances GP (Naghadeh et al., 2020). In the case of grain samples, the GP curve reaches a plateau after 24 or more incubation times (Schiff et al., 2023). Grain processing increases ruminal degradability, resulting in higher GP (Sommart et al., 2000). The higher GP values and kinetics parameters (A, c) in all MF samples indicate high ruminal starch fermentation (Menke and Steingass, 1988) and solubility (Deckardt et al., 2013). Previous studies have reported higher GP and (A, c) values in steam-flaked treatments compared to the control. This can be attributed to the destruction of the protein matrix surrounding starch granules, gelatinization of starch and more fermentation of starch in steam-flaked treatments than the control with a similar particle size (DePeters et al., 2003; Fu qiang et al., 2014).

In contrast, in the WF treatment, the values for GP and the (A, c) parameters were lowest among treatments due to a shift in the site of digestion from the rumen to the small intestine (Karami et al., 2018). In the M treatments, lower GP and kinetics parameters (A, c) were observed due to the denaturation of CP, the Maillard reaction and a reduction in solubility compared to the control (Golshan et al., 2019; Parnian et al., 2013). In all treatments, there was an increase in GP and (A, c) parameters as the particle size decreased. This increase can be attributed to the larger surface area available for microbial degradation, soluble carbohydrate and starch degradation, similar to previous studies (Gallo, 2017; Naghadeh et al., 2020).

The synthesis of MCP requires both available energy and N content (Casper et al., 1999). In our *in vitro* experiment, we found that in addition to the N content in corn, there was an NH_3-N pool in all culture units. Therefore, treatments with higher ruminal OM digestibility would result in higher synthesis of MCP. Owens and Bergen (1983) reported that MCP makes up half of the digested protein in the small intestine. As mentioned earlier, the MF and F samples that were ground showed greater MCP and subsequently higher EUCP than the control. The M samples had higher EUCP than the control, which can be attributed to an increase in the flow of non-degradable protein to the intestine (Anele et al., 2011).

Conclusions

It can be concluded that steam- flaking (with a flake density of 0.45 kg/L) and micronization (plus grinding prior to mixing in concentrate) methods were effective in reducing ruminal digestibility and increasing small intestinal digestibility of starch. However, post-flaking of infrared radiated corn at a flake density of 0.41 kg/L may lead to excessive ruminal degradation above that of steam flaking, increasing the risk of acidosis. Therefore, a flake density greater than 0.41 kg/L may be more appropriate for flaking micronized corn grain to achieve optimal ruminal degradation and maximum small intestine digestibility of starch. We recommend further research to optimize flake density for different varieties of micronized corn grains.

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