IJAS Iranian Journal of Applied Animal Science

Operational Conditions of Micronized Maize Grains Assessed by Modeling Ruminal *in vitro* Gas Production Data and Three Steps Method

Research Article

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Received on: 19 Nov 2020
Revised on: 7 Apr 2021
Accepted on: 1 May 2021
Online Published on: Dec 2021

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Online version is available on: www.ijas.ir

ABSTRACT

Micronization of grains is an energy-consuming heat-based process and reducing treatment time in this method might be economically desirable. Different operational conditions including duration of the process (s), grain surface temperature at the end of the process (˚C), and distance between grain and burner (cm) in a micronizer machine were examined to assess the possibility of reducing processing time using *in vitro* gas production, *in situ* and enzymatic dry matter digestibility of corn grains. Treatments which were made by altering the above operational conditions in order included: Mic 1 (120 s, 155 ˚C, and 35 cm), Mic 2 (60 s, 155 ˚C, and 15 cm), Mic 3 (90 s, 165 ˚C, and 15 cm) and Mic 4 (120 s, 175 ˚C, and 15 cm). There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for micronized corn grains (Mic 1, and Mic 2-Mic 4), respectively. Enzymatic dry matter (DM) digestibility in micronized corn grains (Mic 1=26.46 and Mic 3=29.27) was significantly ($P<0.001$) greater than their representative controls (Raw 1=22.17 and Raw 2=25.68) but increasing severity of the process, enhanced ruminal disappearance of dry matter (66.27 *vs*. 59.32 in Mic 4 and Raw 2, respectively. Out of 11 non-linear tested models, logistic-exponential without lag (LE_0) showed the best performance for fitting gas production results indicated that the extent of gas production may decrease or increase by micronization depending upon the operational conditions. Overall, if the surface temperature of radiated grains at exiting (as a major indicator) is fixed and could be achieved by changing micronizer structure within a shorter time, similar *in vitro* and *in situ* performance in radiated corn grains can be obtained however, overheating may increase risk of ruminal acidosis.

KEY WORDS infrared radiation, grains micronization, non-linear models, small intestine digestibility.

INTRODUCTION

The increasing energetic value of corn grain is a very important economic issue in Asia because out of the top ten major corn importers, five countries are located in this part of the world (FAO, 2018). Although effective rumen starch degradability of corn grain is quite lower than barley and wheat grains (Humer and Zebeli, 2017; Gallo *et al.* 2018), total tract starch digestibility of these grains were almost similar (Ferraretto *et al.* 2013) which might be partial because of large intestine fermentation. Consequently, improving ruminal corn starch degradation through a safe condition for rumen plus maximizing small intestine digestion would be nutritionally desirable (Loy and Lundy, 2019; Ebrahimi, 2020). Micronization is a thermal treatment that includes a rapid increasing the internal temperature of a feed using infrared radiation (IR) with 1.8-3.4 µm wavelength. The penetration of the infrared rays into the sub-

strate within a short time exposure to radiation causes rapid internal heating that vibrates water molecules and could increase gelatinized starch up to about 94% (Fasina *et al.* 1999). On the other hand, denaturation of protein reduced its solubility (Fattah *et al.* 2013) that led to the protection of starch molecules from *in situ* ruminal degradation (McAllister and Sultana, 2011). In the case of barley grain, it was confirmed that ruminal un-degraded starch and protein in micronized seeds digested to a greater extent compared to raw grain under *in vitro* enzymatic small intestine incubation however, such information is not available for digestibility of micronized corn grain. Furthermore, it was found that under *in vitro* rumen fermentation, micronized grain exhibited greater starch digestibility than un-treated sample which challenges *in situ* findings (McAllister and Sultana, 2011).

Unlike steam-flaking in which the duration of steaming for each grain is well standardized and heating is performed with steam (Zinn *et al.* 2002), in the micronization process, heating is a result of radiation of infrared ray and many factors such as processing duration, vibration during micronization, the distance between infrared emitter and seeds conveyer may influence the efficiency of the process, etc. To our knowledge, operational conditions for micronizing maize grain as ruminant feed is not well regulated. We hypothesized that changing processing time and distance between the infrared emitter and seeds conveyer in micronization process may alter *in situ* and *in vitro* enzymatic small intestine dry matter digestibility and *in vitro* gas production kinetic of micronized grains. This study aimed to test the above hypothesis using an automated gas measuring system and appropriate model which was selected by testing various non-linear models.

MATERIALS AND METHODS

The experiments described below were conducted in the central laboratory of Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran.

Micronization of corn grain

Corn grain samples from unknown imported varieties from Brazil were obtained from a nearby private feed mill where Grains were soaked using distilled water and maintained at room temperature (25±2 ˚C) for 6 h before micronization to achieve an initial moisture content of 15%. Whole grain was further exposed to infrared rays with a pilot-scale micronizer (Faravardaneh Ferdowsi Mashhad, Ltd. Mashhad, Iran) which was equipped within natural gas-fired ceramic burner (containing 16 pieces of 17×7.5 ceramics in double rows, maximum thermal capacity was 30 kW) under different operational conditions (Figure 1, supplementary materials).

For this purpose, a single layer of the grain (~3 kg) was spread in the stainless-steel tray (50×124×3 cm) and further placed below the radiation source. The surface temperature of the seeds was measured just before the terminating process using an infrared thermometer (TIR 8863, Terminator, China).

Experimental design and treatment groups

The micronizer manufacturer recommended operational conditions of 120 s, 155 ˚C, and 35 cm for the duration of micronization, the surface temperature at the end of the process, and the distance between seeds and burner, respectively. The first control (Raw 1) and its treatment (Mic 1) were provided by the manufacturer which were prepared from a separate batch of maize grain. The two most important features of all heat treatments which influence the degree of processing are temperature and duration of the application (Zinn *et al.* 2002). Economically, lowering processing time is favorable as less fuel is consumed for the unit of the grain mass. It is found that when the distance between tray and burner was reduced to 15 cm, within 60 s, similar exiting temperature (155 ˚C) was achieved and thus the third and fourth treatments were arranged as Raw2 (second control) and Mic 2 because first control (Raw 1) was not enough for composing other treatments. In the newly developed operational conditions, we increased the duration of micronization to 90 and 120 s and fifth (Mic 3) and sixth (Mic 4) treatments which resulted in surface temperature (at exiting time) of 165 ˚C and 175 ˚C for Mic 3 and Mic 4, respectively. Micronized grains were then cooled to room temperature under an evacuation airflow of 15 m^3/ min at 1 m above the layer of hot grains.

Water absorption index and chemical analysis

Corn grain samples were ground using a hammer mill (Toos shekan, Mashhad, Iran) fitted with a 5 mm sieve for using *in vitro* and *situ* experiments or 1 mm for starch and proximate analysis and stored in two layers plastic bags under room temperature (25±2 ˚C) until further usage. Water absorption index (WAI) of the ground feed samples was measured according to the method described by (Zarzycki *et al.* 2017) and calculated using the following formula:

$$WAI\ (\%) = (W_g/W_{DM}) \times 100$$

Where:

W_g: weight of dry matter of sample plus absorbed water.

W_{DM}: dry matter of original sample before soaking.

All the samples in the present study were assayed in triplicate for dry matter (DM), crude protein (CP), and ether extracts EE by the 934.01, 990.03 and 920.39 methods of

the association of official analytical chemists (AOAC, 2005), respectively. The starch content of samples was determined using perchloric acid method (Rose *et al.* 1991).

In vitro gas production technique

In vitro gas production was carried out based on the method proposed by Menke and Steingass (1988). Accurately, 1000 mg (DM basis±0.02) of the substrates were weighed and transferred to glass bottles (250 mL). Two hours before morning feeding, ruminal content was obtained from two ruminally cannulated Holstein steers fed on a diet of alfalfa hay and concentrate mixture (ratio: 60:40 at 2.5% of body weight).

The rumen contents were collected in two insulated thermo-flasks and immediately transferred to the laboratory, where they were filtered through four layers of cheesecloth to remove the contaminants likely to interfere with the rumen fluid dispensation into the serum bottles. Afterward, the sample contents were preserved at the temperature of 39°C in a water bath under continuous CO_2 flushing to foster an oxygen-free environment. In the morning of incubation, an anaerobic medium was prepared and heated to the temperature of 39°C in the water bath. Following that, 150 ml of the anaerobic media and buffered rumen fluid (ratio: 2:1) was anaerobically dispensed to the pre-warmed oxygen-free serum bottles, purged with CO_2 to remove air from the headspace and sealed by a modified screw cap having O ring rubber in the inner side to prevent the escape of fermentation gas. Incubation was performed in a water bath at the temperature of 39°C for 24 hours. In this process, two additional bottles without substrate were used as blank to assess fermentation potential of buffered rumen liquor.

Gas measurement

An automated computerized gas measurement system (designed and developed in Ferdowsi University of Mashhad) was used to determine the pressure of the produced gas from the substrate fermentation at two-minute intervals. The system was composed of two 16-channel arrays, each of which was equipped with an electric valve. The pressure sensor was connected to the bottle via a 23 cm extension tubes (2 mm internal diameter) that were connected to the cap of bottles using pneumatic arrangements (Figure 2, supplementary materials).

Based on the system adjustments, when the gas pressure reached 5.72 mL, the electric valve would open to completely release the accumulated gas in the headspace within five seconds, after which the valve would close. The amount of produced gas from the substrates was marked by the number of valve openings.

Final pH, _in vitro_ and _in situ_ dry matter disappearances

Final pH in the culture liquid was measured using pH meter (Metrohm 691) immediately after termination of incubation. The equation 2 was used for calculating *in vitro* dry matter digestibility (IVDMD):

IVDMD= (S-(R-B)/S) × 100

Where:
S: weight of substrate (mg).
R: weight of dried residue after *in vitro* incubation (mg).
B: weight of dried residue of representative blanks (mg).

For estimation of *in situ* dry matter digestibility (ISDMD), the nylon bags (12 cm×6 cm with a mean pore size of 45 pm) containing 6 g samples were placed in the rumen of two ruminally cannulated Holstein dairy cows that were fed twice daily (at 08:00 h and 19:00 h) with a ration containing concentrate and forage (40:60) in three replicates for every cow. All bags were removed at the end of the incubation period (12 h) and washed under cold tap water in a washing machine. Then all bags were dried at 60 ˚C for 48 h and weighed to determine ISDMD. One gram of un-degraded rumen residuals was subjected to an enzymatic incubation for 24 h to simulate small intestine digestion (Ngonyamo-Majee *et al.* 2009) and *in vitro* dry matter small intestine was determined.

Modelling the _in vitro_ gas production kinetic

Modelling the *in vitro* gas production kinetic performed using 11 non-linear regression models shown in Table 1. These models were previously developed or used in similar studies (Wang *et al.* 2011). MATLAB software (2020a) was used for fitting each treatment and individual replicate and codes written for fitting the data using LE_0 model are presented in supplementary materials (Table 1).

Evaluation and selection of the models

For evaluating the fitting accuracy of different non-linear models, root mean square error (RMSE) and coefficient of determination (R^2) were calculated as described by Miraei Ashtiani *et al.* (2020) using equations 3 and 4, respectively. Models with lower RMSE and higher R^2 can predict gas production (V_{gp}) as similar as observed values. It should be noted that from the biological point of view when t= 0 then $V_{go}= V_{gp}= 0$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(V_{go} - V_{gp})^2}{n}}$$

$$R^2 = \left[\frac{\sum_{i=1}^{n}(V_{go} - \overline{V_{go}}) \times (V_{gp} - \overline{V_{gp}})}{\sum_{i=1}^{n}(V_{go} - \overline{V_{go}}) \times \sum_{i=1}^{n}(V_{gp} - \overline{V_{gp}})}\right]^2$$

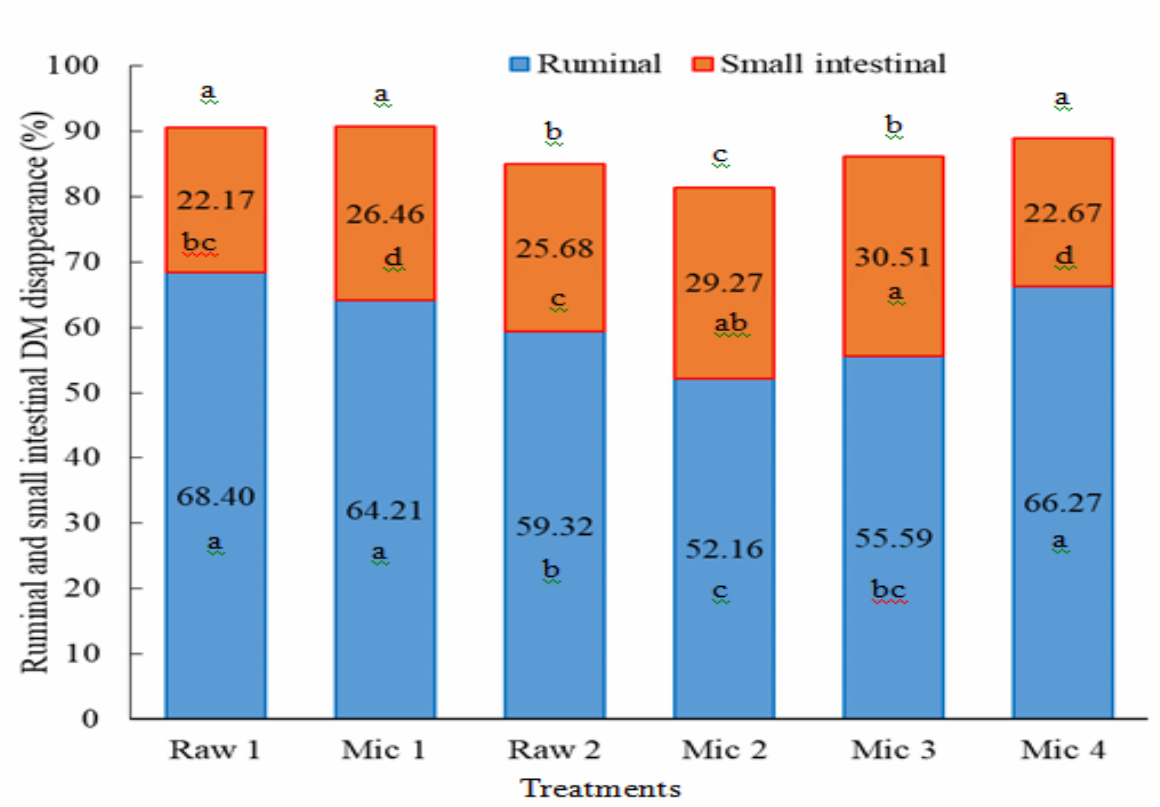

Figure 1 *In situ* ruminal and *in vitro* small intestinal dry matter disappearance of treatments
Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 ˚C, and 35 cm), Mic 2 (60 s, 155 ˚C, and 15 cm), Mic 3 (90 s, 165 ˚C, and 15 cm) and Mic 4 (120 s, 175 ˚C, and 15 cm)
There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic 1 and Mic 2-Mic 4, respectively
a, b, c: bars with different letters differ significantly (P<0.01)
The P-value for ruminal, intestinal, and sum of them was <0.001)
Contrast comparison (Raw *vs*. Mic) was significant for ruminal (P<0.001) and intestinal (P<0.001)
SEM for ruminal= 1.00 and for intestinal= 1.41
SEM: standard error of the means

Statistical analysis

All data were analysed as a completely randomized design using generalized linear model (GLM) procedure of SAS (2004). The employed statistical model was as follows:

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

Where:
Y_{ij}: dependent variable.
μ: overall mean.
T_i: effect of the physical form of the meal.
e_{ij}: residual error.

Orthogonal contrasts were constructed to evaluate the effect of micronization (Mic) versus untreated (Raw) for chemical analysis and *in vitro* measurements.

RESULTS AND DISCUSSION

The chemical composition and WAI of corn grains are presented in Table 2.

It was found that micronization significantly enhanced DM (P<0.001), CP (P=0.01) but reduced starch content (P<0.001) of cooked samples, and increasing processing temperature enhanced these alterations. Žilić *et al*. (2010) observed that micronization decreased crude protein and starch content of corn grain. More studies that statistically compared micronized and raw corn grain were not found, however results of micronization process on the chemical composition of other grains were not conclusive as no effects of micronization on starch, protein, total dietary fibre, ash, and fat of barley grain was reported post micronization (Fasina *et al*. 1999).

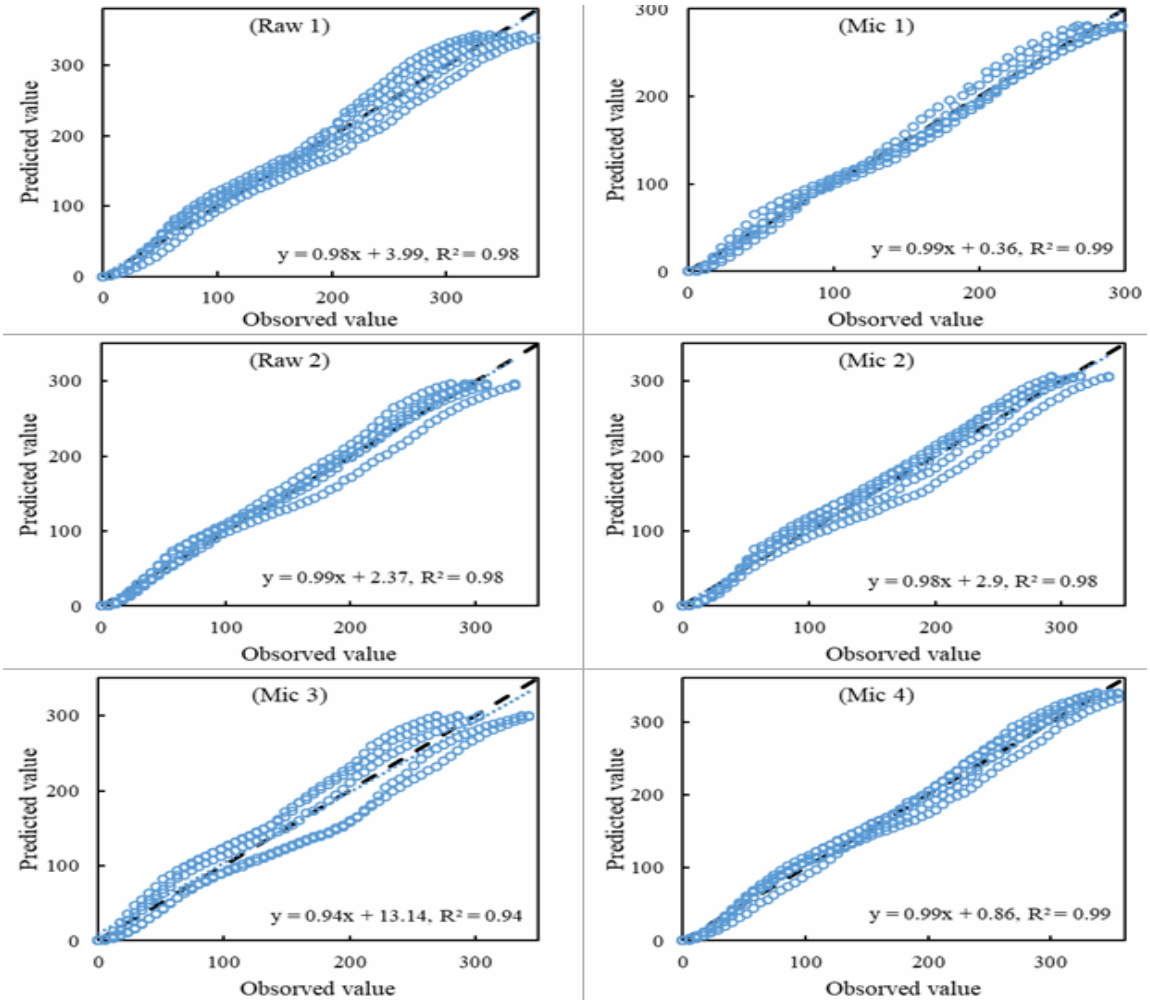

Figure 2 Observed versus predicted values estimated using logistic–exponential without lag time (LE_0)
Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 °C, and 35 cm), Mic 2 (60 s, 155 °C, and 15cm), Mic 3 (90 s, 165 °C, and 15cm) and Mic 4 (120 s, 175 °C, and 15 cm)
There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic1 and Mic 2-Mic 4, respectively

In the case of wheat grain, it was found that the amount of starch, nitrogen, and ether extract was not affected by micronization (Zarkadas and Wiseman, 2001). Niu *et al.* (2003) also reported no effects of micronization on total and gelatinized starch when wheat seeds were micronized for feeding to the broilers. In contrast, micronization of wheat resulted in decrease in starch and an increase in CP content of wheat seeds, respectively (McAllister and Sultana, 2011). Similar to observations of Fasina *et al.* (1999) on barley seeds, infrared heating significantly increased the water absorption index in micronized corn ($P<0.001$) in the current study.

Operational conditions of micronization influenced *in vitro* rumen dry matter digestibility of corn grains (Table 3). Micronization at the lowest processing temperature (155 ˚C) resulted in a significant reduction of IVDMD ($P<0.001$), however, there was a return in DM digestibility values by increasing micronization temperature.

In situ 12 h and 24 h *in vitro* small intestine dry matter disappearance of the treatments used in this study are presented in Figure 1.

Overall, micronization affected both the estimated parameters ($P<0.001$). When the distance of burner and substrate was high (Mic 1), micronization reduced *in situ* DM disappearance from 68.40 in control to 64.21% in processed samples although the difference was not significant. This reduction also significantly occurred when samples were micronized closer to radiation source at 60 s (Mic 2) however at 90 s micronization time (Mic 3), *in situ* dry matter disappearance started to increase and even significantly was greater in grain treated at 120 s (Mic 4) compare to its relative control (Raw 2).

Table 1 Mathematical models used in the current study

Equations[1]	Symbol	Name
$V_g = V_F \frac{t^c}{t^c + K^c}$	MM	Michaelis-Menten
$V_g = V_F \frac{t}{t + K}$	MMM	Modified MM
$V_g = V_F\left(1 - \exp\left(-k(t - LAG) - d(\sqrt{t} - \sqrt{LAG})\right)\right)$	GM	Generalization of the Mitscherlich
$V_g = V_F \frac{1}{1 + \exp(2 + k(\lambda - t))}$	LOG	Logistic model
$V_g = V_F \exp(-\exp(1 - k(t - \lambda)))$	GOM	Gompertz model
$V_g = V_F(1 - \exp(-k(t - LAG)))$	EXP_{LAG}	Exponential model with LAG
$V_g = V_F(1 - \exp(-kt))$	EXP_0	Exponential model without LAG
$V_g = V_F \frac{1 - \exp(-k(t - LAG))}{1 + \exp\left(\ln\left(\frac{1}{d}\right) - k(t - LAG)\right)}$	LE_{LAG}	Logistic-Exponential with LAG
$V_g = V_F \frac{1 - \exp(-kt)}{1 + \exp\left(\ln\left(\frac{1}{d}\right) - kt\right)}$	LE_0	Logistic-Exponential without LAG
$V_g = \sum_{i=1}^{2} V_{Fi}(1 - \exp(-k_i(t - \lambda)))$	TPEXP	Two-pool exponential
$V_g = \sum_{i=1}^{2} V_{Fi} \frac{1}{(1 + \exp(2 - 4k_i(t - \lambda)))}$	TPLOG	Two-pool logistic

V_F: final asymptotic gas volume; t: time; K: the time at $V_F/2$; k: fractional rate of gas production; c and d: shape parameters; LAG: lag time and λ: derived lag time.

Table 2 Chemical composition and water absorption index of raw and micronized corn grains

Items	Treatments[1]						SEM	P-value	P-value
(%)[2]	Raw 1	Mic 1	Raw 2	Mic 2	Mic 3	Mic 4			Raw *vs.* Mic
DM	90.72^{c}	91.86^{b}	89.58^{d}	90.58^{c}	90.98^{c}	92.98^{a}	0.17	< 0.001	< 0.001
CP	9.08^{bc}	9.30^{a}	8.96^{c}	9.04^{bc}	9.11^{b}	9.27^{a}	0.03	< 0.001	0.01
EE	4.09^{a}	4.03^{ab}	4.04^{ab}	4.01^{bc}	3.97^{bc}	3.94^{c}	0.02	< 0.001	0.91
Starch	71.13^{a}	70.82^{bc}	70.91^{b}	70.83^{bc}	70.65^{c}	70.04^{d}	0.05	< 0.001	< 0.001
WAI	230.04^{c}	236.59^{bc}	217.64^{d}	228.13^{cd}	246.04^{b}	266.20^{a}	2.67	< 0.001	< 0.001

1: Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 °C, and 35 cm), Mic 2 (60 s, 155 °C, and 15cm), Mic 3 (90 s, 165 °C, and 15 cm) and Mic 4 (120 s, 175 °C, and 15 cm). There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic 1 and Mic 2-Mic 4, respectively.
DM: dry matter; CP: crude protein; EE: ether extract and WAI: water absorption index.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: Standard error of the means.

Table 3 *In vitro* dry matter digestibility, in situ dry matter degradability and final pH in the culture media of raw and micronized corn grains

Items (%)	Treatments[1]						SEM	P-value	P-value
	Raw 1	Mic 1	Raw 2	Mic 2	Mic 3	Mic 4			Raw *vs.* Mic
IVDMD[2]	91.60[a]	88.12[b]	86.20[b]	79.13[c]	82.59[d]	85.60[b]	0.64	< 0.001	0.006
GP[3]	353.51[a]	285.86[c]	302.6[c]	314.64[bc]	307.85[c]	348.96[ab]	9.35	0.0001	0.05
Final pH	6.34	6.35	6.33	6.35	6.34	6.32	0.007	0.11	0.05

1: Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 °C, and 35 cm), Mic 2 (60 s, 155 °C, and 15cm), Mic 3 (90 s, 165 °C, and 15 cm) and Mic 4 (120 s, 175 °C, and 15 cm). There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic 1 and Mic 2-Mic 4, respectively.
IVDMD: 24 h *in vitro* dry matter digestibility and GP: 24 h *in vitro* gas production (mL/g DM).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: Standard error of the means.

Table 4 Root mean square error (RMSE) and R^2 of different models for treatments

Models	Criteria	Treatments[1]					
		Raw 1	Mic 1	Raw 2	Mic 2	Mic 3	Mic 4
MM	RMSE	16.77	9.32	13.04	14.55	23.50	11.93
	R^2	0.98	0.99	0.98	0.98	0.94	0.99
MMM	RMSE	16.77	9.32	13.04	14.55	23.50	11.93
	R^2	0.98	0.99	0.98	0.98	0.94	0.99
GM	RMSE	21.76	11.97	16.12	19.40	25.43	17.49
	R^2	0.96	0.98	0.97	0.96	0.93	0.97
LOG	RMSE	16.99	10.13	13.44	14.64	24.22	12.20
	R^2	0.97	0.99	0.98	0.98	0.93	0.99
GOM	RMSE	16.16	8.68	12.46	13.92	23.35	10.96
	R^2	0.98	0.99	0.98	0.98	0.94	0.99
EXP_{LAG}	RMSE	21.76	11.97	16.12	19.40	25.43	17.49
	R^2	0.96	0.98	0.97	0.96	0.93	0.97
EXP_0	RMSE	23.16	12.58	16.97	20.67	26.04	19.05
	R^2	0.95	0.98	0.97	0.95	0.92	0.97
LE_{LAG}	RMSE	16.44	9.00	12.67	14.13	23.56	11.30
	R^2	0.98	0.99	0.98	0.98	0.94	0.99
LE_0	RMSE	16.44	9.00	12.67	14.13	23.56	11.30
	R^2	0.98	0.99	0.98	0.98	0.94	0.99
TPEXP	RMSE	25.48	15.47	20.50	21.49	28.08	25.59
	R^2	0.94	0.97	0.95	0.95	0.91	0.94
TPLOG	RMSE	15.52	8.47	12.40	13.53	23.02	10.48
	R^2	0.98	0.99	0.98	0.98	0.94	0.99

1: Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 °C, and 35 cm), Mic 2 (60 s, 155 °C, and 15cm), Mic 3 (90 s, 165 °C, and 15 cm) and Mic 4 (120 s, 175 °C, and 15 cm). There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic 1 and Mic 2-Mic 4, respectively.
MM: michaelis–menten; MMM: modified michaelis–menten; GM: generalized mitscherlich; LOG: logistic; GOM: gompertz; EXP_{LAG}: exponential with lag time; EXP_0: exponential without lag time; LE_{LAG}: logistic–exponential with lag time; LE_0: logistic–exponential without lag time; TPEXP: two–pool exponential and TPLOG: two-pool logistic.

Similar findings for *in vitro* and *situ* confirm that under particular operational conditions, micronization process may reduce ruminal degradation as reported by previous work (McAllister and Sultana, 2011).

The main mechanism for this event is protein denaturation which generally happens by heating (Eckhoff, 2004) because albumins, globulins, prolamins, and glutelins contributed 5, 6, 50, and 39% of total proteins in maize kernels, respectively (Ryšavá, 1994) and denaturation temperatures of albumins, globulins, glutelins and prolamins for extracted from soybean seeds were estimated by 90.62, 81.68, 83.05 and 80.75 ˚C (Makeri *et al.* 2017). Thus it is possible that under the temperature below gelatinization, severe denaturationof maize grain proteins might have occurred. Increasing ruminal dry matter digestibility by enhancing processing temperature could be attributed to greater gelatinization as reported by other studies (Emami *et al.* 2010). Starch gelatinization was not estimated in the current study because there is a positive correlation between starch gelatinization and WAI (Zarzycki *et al.* 2017) and since latter parameter was increased by increasing temperature (Table 2). Micronization of both types of corn grains (Raw 1 and Raw 2) significantly enhanced *in vitro* small intestine dry matter disappearance with an exception of the treatment which was micronized at a close distance to burner (15 cm) at the longest time (120 s, Mic 4).

Under the experimental conditions in the present study, infrared radiation significantly altered location of the digestion from the rumen to small intestine (P<0.001), and the treatment Mic 3 had a significantly similar total disappearance of DM with control (Raw 2) but exhibited greatest small intestine DM disappearance (Figure 1). Our findings are consistent with (Fattah *et al*. 2013) who observed similar enhancement in small intestinal digestibility of ruminal un-degraded micronized barley grain. Therefore, from the last two paragraphs, it can be concluded that firstly, in the micronization process there is optimum operational condition that ruminal degradation may reduce at the benefit of increasing small intestine digestibility. Secondly, changing operational conditions and machine structure for obtaining similar surface temperature at exiting within a shorter time caused an almost equivalent reduction in ruminal and increase in small intestine digestibility of micronized corn grains compare to the un-heated substances.

No effect of micronization was found on final *in vitro* ruminal pH however, a significant reduction of *in vitro* 24 h gas production in micronized corn (Mic 1) compared to raw one (Raw 1) was observed (Table 3). This indicates that *in vitro* DM digestibility of micronized grains was in line with those above *in situ* MD disappearance which was because of similar particle grains size in substrates used for two procedures of *in vitro* and *in situ*. McAllister and Sultana (2011) reported that when micronized grain was ground quite fine for *in vitro* method, the effect of micronization on reducing DM digestibility was omitted and opposite results were found with *in situ* test.

Table 4 represents RMSE and R^2 of different models. As shown, TPLOG model resulted in the lowest RMSE values for all groups of treatments however the highest values of RMSE were observed in the TPEXP model which indicated TPLOG and TPEXP models had maximum and minimum ability to predict gas production across all the treatments. Performance of LE_0 and LE_{LAG} indicated that presence of LAG parameters in the model had no strong effect on the model performance and therefore it could be omitted from the model.

Similarly, there was no advantage of the existing of c parameter in MMM model compare to MM model. Accordingly, TPLOG, GOM, LE_0, and MMM were the best candidates for later assessment based on their lower and higher RMSE and R^2, respectively.

P-values of statistical comparison between observed and predicted data set for mean (P_M), variance (P_V), and distribution (P_D) and also the minimum value of predicted data (Min_P) for candidate models in different treatments are presented in Table 5.

It appears that all models could generate data that did not significantly differ from observed values in mean, variance, and statistical distribution as all P-values were greater than 0.01. Although P-values of TPLOG and GOM models were greater than two other models but Min_P in these models were greater than zero.

At the beginning of incubation (zero time), there was no gas production therefore both TPLOG and GOM models which have non-zero minimum value of predicted data are facing with this weakness point.

Table 5 Comparison of selected models within different treatments

Models[2]	Criteria[3]	Treatments[1]					
		Raw 1	Mic 1	Raw 2	Mic 2	Mic 3	Mic 4
TPLOG	P_M	0.98	0.97	0.97	0.98	0.98	0.98
	P_V	0.81	0.87	0.81	0.81	0.56	0.87
	P_D	0.86	0.75	0.80	0.81	0.80	0.86
	Min_p	16	15.35	15	14.18	15.23	16.50
GOM	P_M	0.97	0.99	0.99	0.97	0.99	0.98
	P_V	0.91	0.93	0.89	0.93	0.60	0.98
	P_D	0.81	0.97	0.99	0.96	0.52	0.99
	Min_p	5.26	7.75	6.91	4.84	6.86	6.28
LE_0	P_M	0.96	0.97	0.97	0.96	0.99	0.96
	P_V	0.91	0.99	0.95	0.94	0.61	0.99
	P_D	0.61	0.97	0.86	0.75	0.52	0.95
	Min_p	0	0	0	0	0	0
MMM	P_M	0.86	0.91	0.90	0.87	0.91	0.87
	P_V	0.85	0.85	0.89	0.84	0.80	0.78
	P_D	0.61	0.88	0.73	0.61	0.80	0.67
	Min_p	0	0	0	0	0	0

1: Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 °C, and 35 cm), Mic 2 (60 s, 155 °C, and 15cm), Mic 3 (90 s, 165 °C, and 15 cm) and Mic 4 (120 s, 175 °C, and 15 cm). There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic 1 and Mic 2-Mic 4, respectively.
TPLOG: two-pool logistic; GOM: gompertz; LE_0: logistic–exponential without lag time and MMM: modified michaelis–menten.
3: P_M, P_V, P_D, and Min_p: P-values of statistical comparison between observed and predicted data set for mean (P_M), variance (P_V) and distribution (P_D) and also minimum value of predicted data (Min_P).

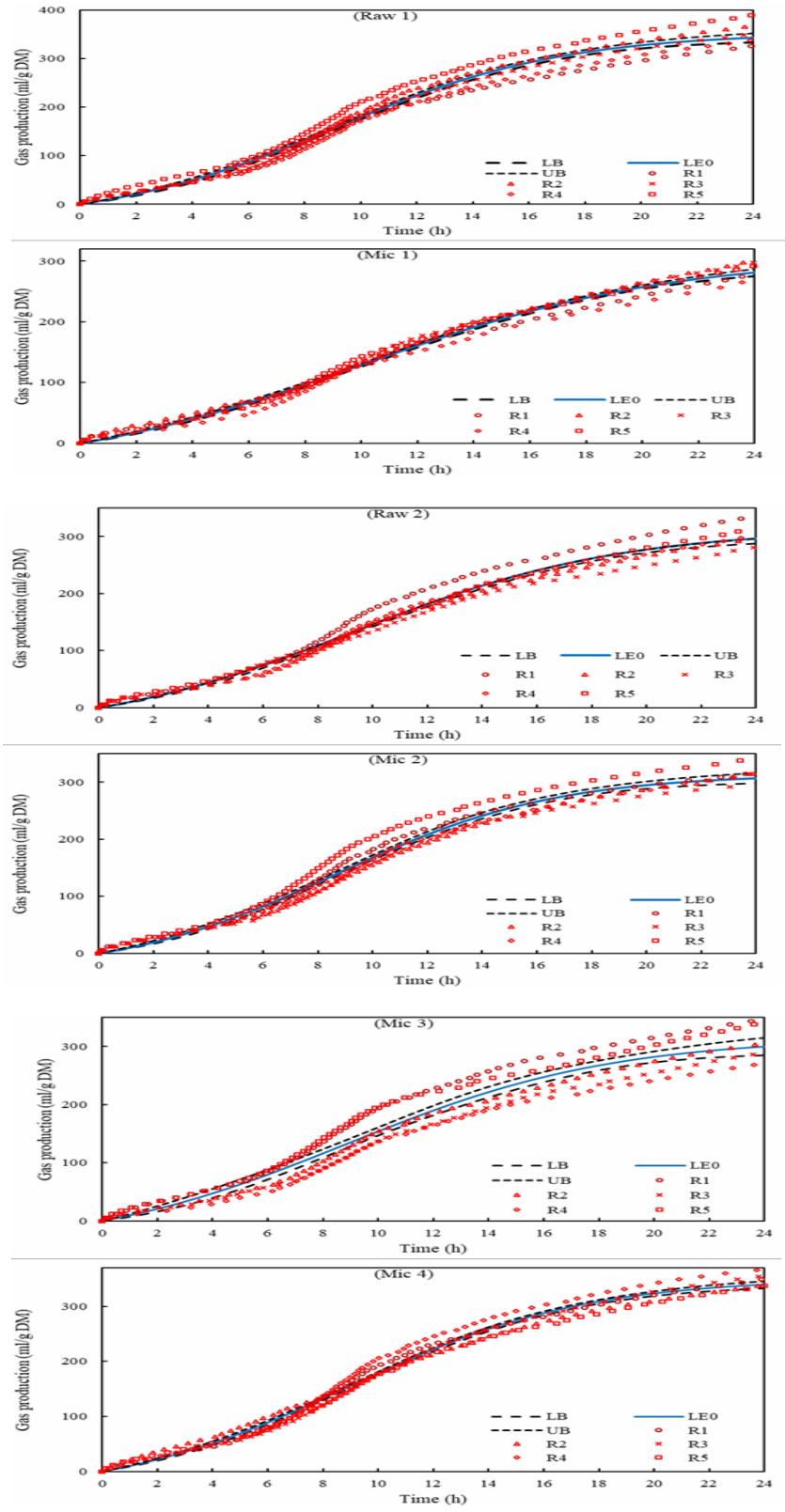

Figure 3 Pattern of 24 h gas production fitted by Logistic–Exponential without lag time (LE_0) using five replicates (R1-R5) in different treatments

Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic 1 (120 s, 155 °C and 35 cm), Mic 2 (60 s, 155 °C and 15cm), Mic 3 (90 s, 165 °C and 15 cm) and Mic 4 (120 s, 175 °C and 15 cm)

There were two batches of raw corn which were included Raw 1 and Raw 2 as control for Mic 1 and Mic 2-Mic 4, respectively

R1-5 indicate replicates for each treatment

LB and UB represent lower and upper bound

Table 6 Estimated parameters of Logistic–Exponential model without lag time (LE_0)

Parameters	Treatments[1]						SEM	P-value	P-value
	Raw 1	Mic 1	Raw 2	Mic 2	Mic 3	Mic 4			Raw *vs.* Mic
V_F	353.26^{a}	309.18^{b}	314.7^{b}	312.52^{b}	310.70^{b}	353.44^{a}	9.98	0.004	0.01
k	0.24^{ab}	0.17^{c}	0.21^{bc}	0.27^{a}	0.2b^{c}	0.23^{ab}	0.02	0.008	0.002
d	0.11	0.18	0.16	0.1	0.14	0.14	0.03	0.26	0.17
R^2	0.98	0.99	0.98	0.98	0.94	0.99	-	-	-
R^2_{adj}	0.98	0.99	0.98	0.98	0.94	0.99	-	-	-

1: Mic 1-4 represent micronized corn grain under different operational condition (duration of micronization, surface temperature at the end of the process and distance between tray and burner) as follow: Mic1 (120 s, 155 °C, and 35 cm), Mic 2 (60 s, 155 °C, and 15cm), Mic 3 (90 s, 165 °C, and 15 cm) and Mic 4 (120 s, 175 °C, and 15 cm). There were two batches of raw corn which were included Raw 1 and Raw 2 as the control for Mic 1 and Mic 2-Mic 4, respectively.
V_F: final asymptotic gas volume; k: fractional rate of gas production and d: shape parameter.
Values represent means ± standard error of five replicates and means bearing different superscript letters differ significantly (fisher LSD method and 95% confidence).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: Standard error of the means.

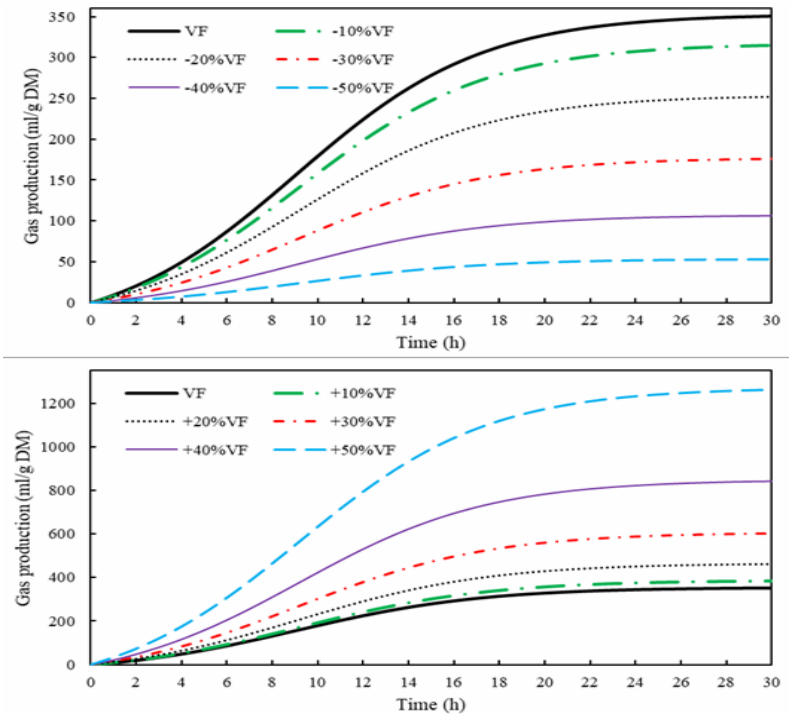

Figure 4 Sensitivity analysis of Logistic–Exponential without lag time (LE_0) with reducing (-) or increasing (+) V_F parameter in the Raw 1 (Raw corn grain) treatment

Two remained models (LE_0 and MMM) had zero Min_P however, according to Table 4, LE_0 model exhibited lower RMSE and greater R^2 than MMM model and could be selected as the best model for describing gas production kinetic of raw as well as micronized maize grains.

Although LE_0 was first developed for interpreting 15 gas data points at fixed incubation times (Wang *et al.* 2011) but Muetzel *et al.* (2014) using an automated gas measuring instrument and 20-25 measurements of gas volume at non fixed times found capabilities of this model to describe gas production kinetics which is line with our findings that had up to 70 data points. Regression estimates which were used to explain the relationship between observed and predicted values using LE_0 model are illustrated in Figure 2.

In all the treatments, R^2 of regression equations are equal to 0.94 and greater with slopes near to 1 (ranging from 0.94-0.99) which confirmed the validity of LE_0 model. Figure 2 also shows model performance by using the selected model (LE_0) and five replicates of different treatments. As presented, the sigmoid function passed well through replicates as lower and upper 95% confidence bound were small enough. Data of the first treatment (Raw 1) were used to analyze sensitivity of the selected model against 10, 20, 30, 40 and 50% decrease or increase in model parameters relative to their initial estimated values (V_F=352.80, k=0.24, and d=0.11) and results are demonstrated in Figures 4-6. As shown in Figure 4, enhancing of V_F parameter resulted in a decrease or increase in gas production however, there was

no notable change up to 20%. Furthermore, alterations of V_F parameter could affect only the amount of gas production and not the curve shape or pattern of gas production. Reducing k parameter decreased the slope of the curve and vice versa (Figure 5) but the effects on the amount gas production erewere small up to the ±10% change in the k value. Sensitivity analysis determined that d parameter is more sensitive to increasing than reduction (Figure 6).

Parameters of the selected LE_0 model for the raw and micronized maize grains are summarized in Table 6. High values of R^2 and adjusted R^2 evidenced sufficiency of LE_0 for predicting 24 h *in vitro* ruminal gas production kinetic parameters of maize grain. Micronization of maize grain (Raw 1) for two min at 35 cm distance between burner and substance which could increase the temperature of grain surface up to 155 ˚C significantly reduced both V_F and k parameters (P=0.004) indicating the impact of micronization on decreasing ruminal digestion as explained above however changing operational conditions acted differently on estimated parameters because severe micronization (reducing the distance to 15 cm at the similar time (2 min) resulted in surface temperate of 175 ˚C, Mic 4) caused an increase in V_F which was un-expectable event to compare with whatever that happened in Mic 1.

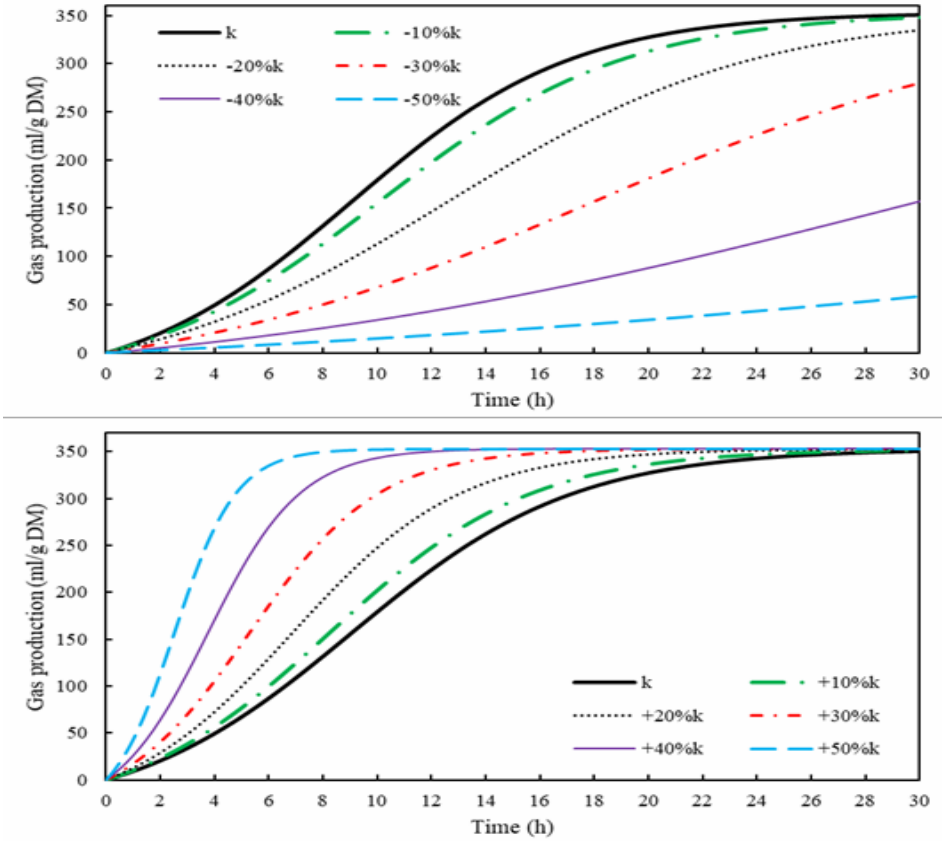

Figure 5 Sensitivity analysis of Logistic–Exponential without lag time (LE_0) with reducing (-) or increasing (+) k parameter in the Raw 1 (Raw corn grain) treatment

Figure 4 also illustrates extent and rate of gas production obtained using the selected model for raw and processed maize grains. Instantaneous rate of gas production indicated that in the first 12 h of incubation depending upon the type of raw grain and processing condition, different effect of micronization on rate of gas production was observed (Figure 7).

Despite a marked decrease in the rate of gas production during initial times of incubation in micronized grain (Mic 1), micronizing Raw 2 corn grain resulted in increase in the instantaneous rate of gas production in the first 12 h of incubation (Mic 4).

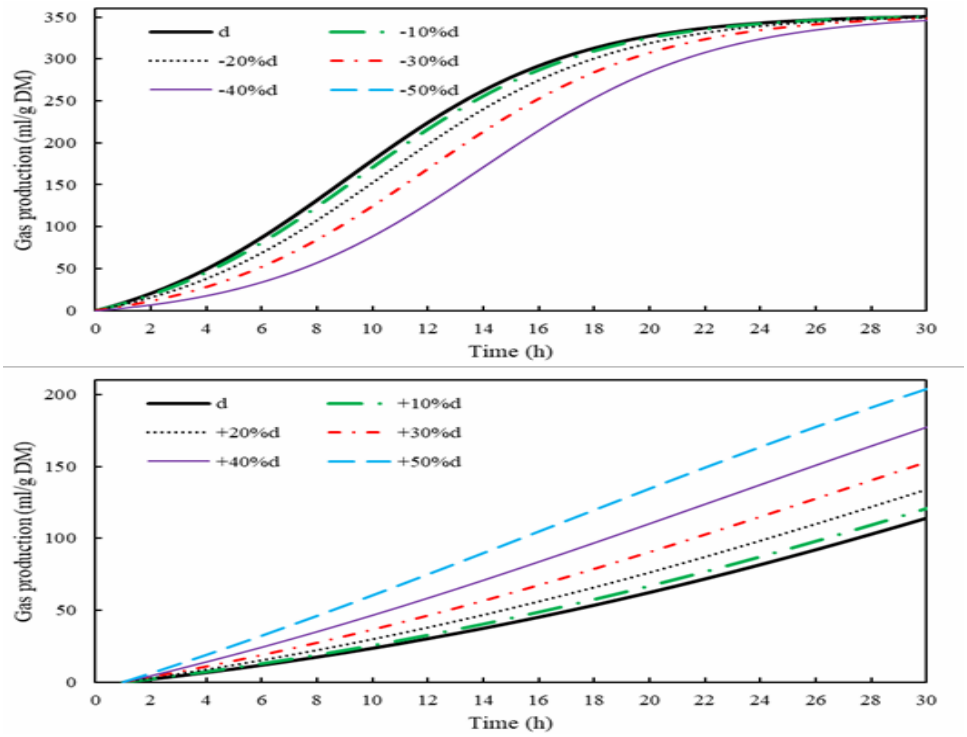

Figure 6 Sensitivity analysis of Logistic–Exponential without lag time (LE_0) with reducing (-) or increasing (+) d parameter in the Raw 1 (Raw corn grain) treatment

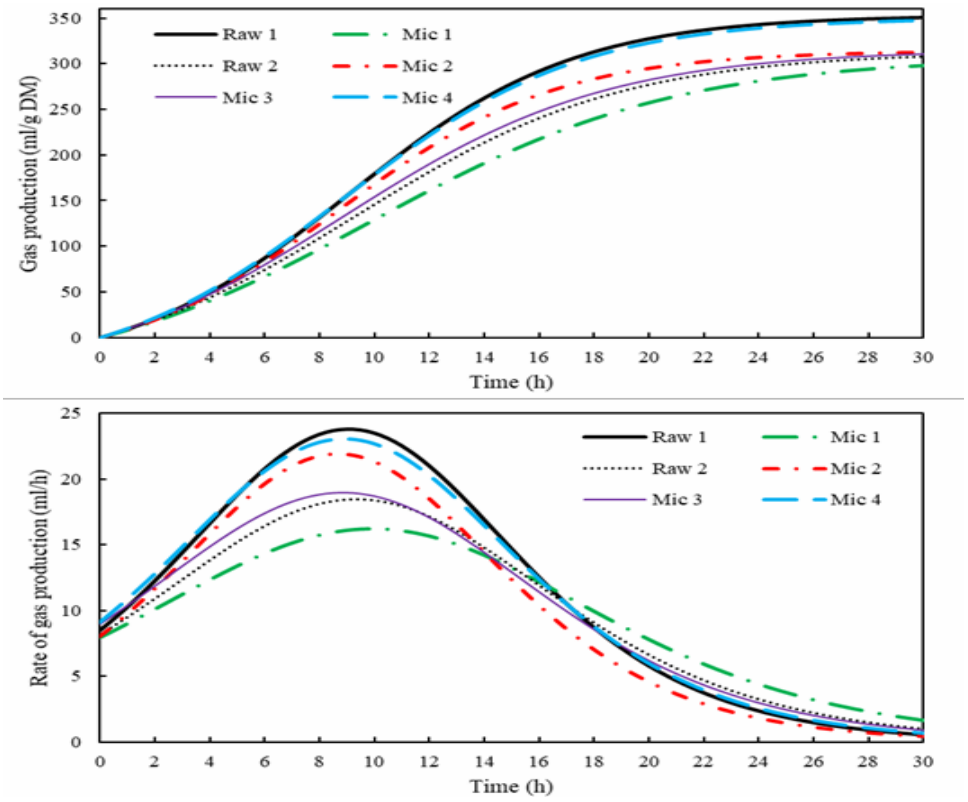

Figure 7 Extent and rate of gas production in experimental treatments obtained using Logistic–Exponential without lag time (LE_0)

It would be possible that an incomplete gelatinization happened in Mic 1 which had lower ruminal and slightly higher small intestinal dry matter disappearance compared to Raw 1. In the other micronized treatments (Mic 2-4), the degree of starch gelatinization as appeared in the water absorption index was more and balanced which could result in greater small intestinal dry matter disappearance in the Mic 3 treatment. Therefore, micronization do not guarantee lower acidosis rate as claimed by McAllister and Sultana (2011) because its effect are not fixed and depends on the operational conditions.

CONCLUSION

Overall, it appears from the results of the present study that surface temperature at exiting from the micronizer is an appropriate indicator that determines the sufficiency of the process. Furthermore, overheating may result in sever ruminal degradation which may not be desirable when high levels of grains are going to be fed to the ruminant. Results confirm that grains temperature of 155 ˚C at exiting of micronizer can be established as maximum temperature capable to change DM digestibility change from the rumen to small intestine. This processing method probably requires a balance between quantities of two events during heat treatment: denaturation of proteins and starch gelatinization. Therefore, for every micronizer system depending upon the structure, the best operational condition for target species (ruminant or non-ruminant) must be standardized. Another independent outcome of the current work was also exploring the capability of Logistic-Exponential without LAG model (LE_0) to fit gas production data measured using automated gas measuring system obtained from 24 h *in vitro* incubation of high energy substrate.

ACKNOWLEDGEMENT

This study is supported by a grant from Ferdowsi University of Mashhad (N. 2/50550). The authors thank Faravardaneh Ferdowsi Mashhad for providing micronizer flaker machine and technical assistance for preparing treatments. We also appreciate given helps by Miss Hanieh Sajjadi, Maryam Roshandel, Hamed Alipour, and Omid Rabbanizadeh for farm and laboratory assistance. There are no relevant financial or non-financial competing interests to report.

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