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RESEARCH ARTICLE

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Ruminal and post-ruminal barley grain digestion and starch granule morphology under three heat methods

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Abstract

This study was designed to investigate the effect of three heating methods on the ruminal and post-ruminal nutrient degradability and starch granule morphology of barley grain (BG - *Hordeum vulgare*). Treatments were Control (CG): without processing; Roasted (RG): roasted BG for 300 s at 130°C; Microwaved (MG): irradiated BG for 120 s at 1200 W; and Steam flaked (SG): BG misted for 30 min under steam flow of boiling water and flaked. Gas production and in situ techniques were used to evaluate the ruminal degradability of treatments, and a modified three-step method was utilised to estimate the total-tract digestibility. Morphological changes of starch granules were determined by field emission scanning electron microscopy (FESEM). Ruminal gas production and dry matter disappearance were increased ($p < .05$) in SG *vs.* CG. Heat processing had different effects on starch and crude protein degradability; however, starch degradability increased ($p < .05$) from CG to SG. Post-ruminal disappearance of dry matter in CG was greater ($p < .05$) than other treatments. These results validated by FESEM images that explained high barley grain degradability in relation to the number of holes on the surface of starch granules. Heat processing can enhance ruminal and post-ruminal utilisation efficiency of barley grain, resulting in improvement of total-tract digestibility.

KEYWORDS

barley, degradability, gas production, heat methods, starch

1 | INTRODUCTION

One of the major purposes of ruminant feeding management is to predict better performance based on chemical composition and feedstuff ingredients (Dehghan-Banadaky et al., 2007). To meet high performance dairy cattle energy requirements, barley grain is widely used, which can perform as well as corn grain when it is hull-less (Giuberti et al., 2014; Yang et al., 2017). Several studies mention that grain processing and mean particle size are major factors in microbial protein synthesis and ruminal degradability of starch (Ferraretto et al., 2013; Yang et al., 2000; Zhao et al., 2016). Factors such as chemical composition, morphology and amount of crystallisation of starch granules, endosperm vitreousness, the ratio of amylose to amylopectin, and the presence of a complex of lipid amylose covering the starch granules affect the digestion dynamics of starch (Giuberti et al., 2014). Processing improves apparent digestibility of dry matter, protein and starch and milk yield, but decreases fine particle size (Ahmad et al., 2010; Yan et al., 2014). Improved ruminal N utilisation

List of Abbreviations: AS, acetone solutions; ADF, acid detergent fibre; AOAC, Association of Official Analytical Chemists; BG, barley grain; CNCPS, Cornell net carbohydrate and protein system; CA, crude ash; CP, crude protein; DOM, digestible organic matter; DM, dry matter; EE, ether extract; FESEM, field emission scanning electron microscope; GP, gas production; ME, metabolizable energy; MG, microwave irradiated barley grain; NRC, National Research Council; NDF, neutral detergent fibre; N, nitrogen; RECANR, Research and Education Center for Agriculture and Natural Resources; RG, roasted barley grain; SCFA, short chain fatty acid; SEM, standard error of mean; SG, steam flaked barley grain; TTD, total tract digestibility; VFA, volatile fatty acids.

and reduced energy loss via decreasing methane production can be considered the advantages of heat processing (Zinn, 1993). Cereal starch cannot be digested completely in the rumen, but it is a widely known carbohydrate source used for ruminants. Because the intact pericarp of barley grain is resistant to bacterial attachment and ruminal digestion, barley grain should be processed before feeding to expose the endosperm to the ruminal microbial population (Dehghan-Banadaky et al., 2007; McAllister et al., 1994). Steam rolling of grain reduces fine particles and synchronisation of heat and moisture causes gelatinization of starch granules, and roasting may reduce ruminal digestibility of starch, but escaped starch from the rumen can increase the energy efficiency (Dehghan-Banadaky et al., 2007). Microwave irradiation of grains can be effective in destroying the protein matrix of covered granules, exposing them to microbial and enzymatic digestion (Khajehdizaj et al., 2014). In order to determine the digestibility and ruminal degradation extent of feed stuffs, several in vitro and in situ methods were developed, which can help us to study the different responses to variations in rumen inoculum (López, 2005). Using new technologies leads us to more precise data, compared with old methods, in revealing information related to natural phenomena. The field emission scanning electron microscope can offer new insight into ways of enhancing digestibility, by providing not only more zoom range and resolution, but also more field of depth, which aids observation of rugged, perforated, covered surfaces and topography of objects, especially biological ones. Limited data are available about images of starch contribution to digestibility with special processing. Therefore, the purpose of the present study was to introduce nano-technology instrumentation as a new assay for illustrating the morphology and kinetics of digestion induced by the number of digestion orifices made by the rumen biomass, digestion barriers and their obviation in barley grain.

2 | MATERIALS AND METHODS

2.1 | Animals and feeding

All animal studies followed the Iranian Council on Animal Care guidelines (1995). Three cannulated adult Ghezel wethers with live weights of 50 ± 3 kg were used to obtain rumen liquor and to estimate in situ dry matter, crude protein and starch degradation. Wethers were fed twice a day. The total mixed ration composition formulated with CNCPS Sheep version 1.0.21 was 270 g kg^{-1} alfalfa hay, 302 g kg^{-1} barley straw and 284 g kg^{-1} concentrate including dry-rolled barley grain, ground corn grain, soybean meal, wheat bran, mineral vitamin premix and salt (DM basis) in equal meals at the maintenance level. Animals had free access to fresh water (NRC, 2007).

2.2 | Samples preparation and treatments

Pure-bred CB-74-2 variety of barley samples were collected from the East Azerbaijan Research and Education Center for Agriculture and Natural Resources (RECANR). Treatments were (a) control: barley grain without processing (CG), (b) roasted: grains roasted (Roasting Titan EF-6100 CE 220 V, 50–60 Hz, 1,400 watt) for 300 s at 130°C (RG), (c) microwaved: grains irradiated in a Pyrex pan in which the height was not more than 1–2 cm for 120 s at 1200 W (MG), and (d) steam flaked: grains misted for 30 min in direct steam flow of boiling water and flaked (SG).

2.3 | Chemical analysis

Samples (n = 3) were dried in an oven at 135°C for 2 hr and the DM was calculated (AOAC, 2005; Method 930.15). Samples were analysed for ash at 600°C for 2 hr (AOAC, 2005; Method 942.05), N content was determined using Kjeldahl (Foss Electric, Copenhagen, Denmark) (AOAC, 2005; Method 984.13). Cell-wall content of samples (acid detergent fibre (ADF) and Neutral detergent fibre (NDF)) were determined, using Van Soest et al. (1991) assay, in which a heat-stable α-amylase Sigma (Number A3306, Sigma Chemical Co., St. Louis, MO) was utilised. Ether extract (EE) content was extracted with ether (AOAC, 2005; Method 920.39). The starch content of the samples was measured using a spectrophotometer in the wavelength of 630 nm with Anthrone reagent (Hedge et al., 1962).

2.4 | In vitro Gas production

Samples of both treated and untreated barley grains were ground in a Wiley Mill adjusted to 2 mm screening, 300 mg of each sample was weighed into 50 mL volume glass vials, samples and prepared buffer

TABLE 1 Chemical composition (%, mean ± *SD*)[a] of treated and untreated barley grain treatments

Barley grain	DM	CP	NDF	ADF	EE	Ash
CG	90.53 ± 3.105[b]	9.91 ± 1.503	20.73 ± 1.101	6.13 ± 0.350	3.87 ± 0.490	1.93 ± 0.163
RG	91.19 ± 2.816[ab]	10.23 ± 0.800	21.06 ± 1.321	6.89 ± 1.052	3.07 ± 1.022	1.87 ± 0.181
MG	93.00 ± 2.717[a]	10.11 ± 0.991	22.00 ± 1.117	7.35 ± 0.353	3.69 ± 0.341	2.02 ± 0.120
SG	91.38 ± 0.103[ab]	11.02 ± 0.211	21.73 ± 0.308	7.53 ± 0.309	3.57 ± 0.501	2.20 ± 0.316
NRC 2001	91.0	12.4	20.8	7.2	2.2	1.9

[a–b]Means within a column with different superscripts differ (p < .05).
[a]DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract (mean ± *SD*); p-value = .0001).
[b]CG, whole barley grain; RG, roasted barley grain; MG, microwave-irradiated barley grain; SG, steam-flaked barley grain.

(McDougall, 1948) were kept at 39°C in a water bath. Rumen fluids from the three cannulated adult wethers were obtained 2 h after morning feeding and all were mixed in the same proportion. Every treatment sample was incubated in six replicates of the digestion medium contained rumen liquor and buffer solution (1:2 vol/vol) and six blank vials were filled with only digestion medium. The experimental vials were placed in a shaker platform after sealing and adjusted to 39°C. Gas production data for each vial were recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 hr of incubation according to Fedorah and Hrudey (1983).

2.5 | In situ ruminal degradability

Three wethers fitted with rumen cannulas were used for in situ procedures. Wethers were adapted to the diet at the maintenance level for 14 days before starting the in-situ incubation; wethers had free access to fresh water. Rumen digestion kinetics of both treated and untreated barley grains were estimated using the in-situ method. Briefly, 5 g dry matter (DM) of sample ground in a Wiley Mill adjusted to 2 mm screening was weighed into bags (60 mm × 120 mm) made of polyester (pore size of 50 ± 4 μm). Nylon bags were sealed with glue and placed in a larger (15 cm × 15 cm) mesh bag prior to incubation in the rumen. Two sample bags were placed in the rumen of each wether for every incubation time of 0, 4, 8, 12, 24, 36 and 48 hr. After each time, sample bags were rinsed under running faucet water until the sullage was limpid, and then the bags were oven dried for 48 hr at 60°C. The same washing method was used for zero-hour bags, and DM, crude protein (CP) and starch disappearance were estimated.

2.6 | In vitro intestinal digestion

To evaluate in vitro intestinal digestibility of DM, a modified three-step method (Gargallo et al., 2006) was used. Ruminal residues of treatments incubated for 12 hr with the rumen of the three wethers in nylon bags were washed in tap water until the water was clear. Polyester bags were oven dried for 48 hr at 60°C and pooled by animal and sample. Dried samples (0.5 g DM) were weighed into 5 cm × 5 cm polyester (pore size of 50 ± 3 μm) bags. Every run had three empty bags as blanks. Bags were incubated in the incubation solution contained hydrochloric acid (0.1 M, pH of 1.9 at 39°C) and pepsin (1 g L^{-1} of P-7000 [Sigma, St. Louis, Missouri]) for 60 min with a steady rotation at 39°C in a Daisy incubator. Bags were then washed in cold faucet water. The bags were re-incubated at 39°C for 24 hr with 120 RPM in the incubation bottles containing pre-warmed pancreatin medium according to Mesgaran and Stern (2005). Sample bags were then oven dried for 48 hr at 60°C. Weights of the bags and their contents were recorded.

2.7 | Field emission scanning electron microscope

To ensure that incubation of the treatments in the rumen was similar to previous tests, bags for the field emission scanning electron

TABLE 2 Cumulative gas production (mean ± *SD*) of treated and untreated barley grain (mL g^{-1} of DM)[a]

Barley grain	Hours											
	2	4	6	8	12	16	24	36	48	72	96
CG	25.2 ± 0.61[b]	54.7 ± 0.39[d]	84.0 ± 0.40[d]	116.0 ± 1.16[d]	150.2 ± 1.25[d]	180.0 ± 1.39[d]	208.5 ± 1.21[d]	231.0 ± 1.08[d]	244.5 ± 1.45[d]	252.0 ± 1.62[c]	253.8 ± 1.34[d]
RG	25.4 ± 0.33[b]	56.0 ± 0.49[c]	90.4 ± 0.47[c]	128.3 ± 0.60[c]	167.8 ± 0.80[c]	205.6 ± 1.10[c]	240.1 ± 1.08[c]	265.8 ± 1.05[c]	280.3 ± 1.06[c]	287.8 ± 1.01[c]	290.1 ± 1.14[c]
MG	26.6 ± 0.47[a]	59.8 ± 0.57[b]	97.8 ± 1.19[b]	137.3 ± 0.85[b]	179.3 ± 1.18[b]	216.6 ± 1.14[b]	247.5 ± 1.16[b]	271.8 ± 1.15[b]	287.5 ± 1.65[b]	296.3 ± 1.37[b]	299.4 ± 1.10[b]
SG	27.0 ± 0.38[a]	60.9 ± 0.16[a]	100.3 ± 0.92[a]	138.9 ± 1.59[a]	183.3 ± 1.55[a]	220.1 ± 1.95[a]	253.6 ± 2.29[a]	278.9 ± 2.25[a]	296.7 ± 2.30[a]	306.5 ± 1.99[a]	310.3 ± 2.13[a]

[a–d]Means within a column with different superscripts differ ($p < .05$).

[a]CG, whole barley grain; RG, roasted barley grain; MG, microwave-irradiated barley grain; SG, steam-flaked barley grain (mean ± *SD*); *p*-value = .0001).

microscope (FESEM) procedure were incubated in the rumen for 4, 8, 12, 24, 36 and 48 hr; 0 hr bags were treated as in the in situ procedure. But there were some changes, after each incubation time. Bags were rinsed under running faucet water until the sullage was limpid. Samples were fixed using 4% glutaraldehyde at 4°C for overnight. After fixation, the samples were washed with buffer (4% Glutaraldehyde in 2 M Sodium cacodylate) for 15 min, then serially dehydrated using 30, 50, 70, 80, 90 and 100% acetone solutions for 15 min at each dilution, but the procedure was repeated for 100% acetone solutions for 15 min after dehydration (Parakhia, 2017). Samples were oven dried at 38°C for 30 min. Sub-samples of each treatment were collected and placed in a sputter coater for gold-platinum coating (process current 10 mA for 2 min) and then FESEM (MIRA3 TESCAN) photos were taken at the same scale and same magnification for all samples.

2.8 | Calculations and statistical analyses

Collected data were evaluated in a complete randomised design using SAS software (version 9.1, the ANOVA procedure) and the *SD* was considered for differences between means. Kinetics of digestion in the gas production procedure were described using the model of GP = $A(1-e^{-ct})$. The metabolizable energy (ME, MJ kg^{-1} DM) content of treatments was calculated according to the equation of Getachew et al. (2002), while the DOM (Digestible organic matter), and SCFA (Short chain fatty acids) were calculated using the equations of Menke (1988).

Degradation kinetics of DM, CP and starch were calculated using the following model:

$$y = a + b\left(1 - e^{-ct}\right)$$

3 | RESULTS

3.1 | Chemical composition

A significant difference ($p < .05$) was found between the dry matter (DM) content of the experimental treatments, whereas the MG had the highest and CG had the lowest DM values. However, no significant differences were observed for other nutrients (Table 1).

3.2 | Gas production

Steam-flaked grain samples (*i.e.*, SG) showed greater ($p < .05$) gas production *vs.* other treatments; however, the lowest value belonged to control samples (Table 2). The differences ($p < .05$) between the estimated values of ME, SCFA and OMD, which observed among treatments, can be related to variation in gas produced during 24 hr. Gas production parameters (*A* and *c*) were highest in SG and lowest ($p < .05$) in CG *vs.* other treatments (Table 3).

3.3 | Ruminal degradability

Heat processing had different effects ($p < .05$) on starch and crude protein degradability; starch degradability increased from 535 (for CG) to 569, 692 and 747 g kg^{-1} DM (for RG, MG and SG), respectively. The CP degradability decreased ($p < .05$) from 555 (for CG) to 514, 484 and 444 g kg^{-1} DM (for RG, MG and SG), respectively (Table 4).

3.4 | Intestinal digestion

The digestibility showed an increase ($p < .05$) after 12 hr of ruminal incubation for treated barley grain, whereas post-ruminal digestibilities decreased ($p < .05$) *vs.* untreated grain. However, untreated barley grain and SG had lower and higher ($p < .05$) ruminal DM disappearance, respectively, *vs.* other treatments (Table 5).

3.5 | Field emission scanning electron microscope

To make a good comparison and avoid destruction of granules, all photos were taken by the field emission scanning electron microscope (FESEM) in the magnification range of 5 kX and 5 kV, and the secondary detector from the starch granule surface was focused on the protein matrix and holes created by the rumen biomass. According to the photos in Figures 1 to 4, protein matrices surrounded the starch granules and limited the access of amylolytic microbes to the starch granules. Mild to severe processing methods of RG, MG and SG showed a

TABLE 3 In vitro gas production parameters (mean ± *SD*)[a] of treated and untreated barley grain[b]

Barley grain	ME	SCFA	DOM	A	c
CG	9.12 ± 0.012[d]	0.92 ± 0.005[d]	512.8 ± 0.06[d]	250.94 ± 0.056[d]	0.0801 ± 0.0002[c]
RG	9.98 ± 0.005[c]	1.06 ± 0.001[c]	576.1 ± 0.10[c]	287.85 ± 0.063[c]	0.0801 ± 0.0003[c]
MG	10.33 ± 0.007[b]	1.09 ± 0.004[b]	591.0 ± 0.10[b]	294.64 ± 0.108[b]	0.0815 ± 0.0001[b]
SG	10.53 ± 0.020[a]	1.12 ± 0.001[a]	603.6 ± 0.15[a]	304.83 ± 0.032[a]	0.0845 ± 0.0006[a]

[a–d]Means within a column with different superscripts differ ($p < .05$).
[a]CG, whole barley grain; RG, roasted barley grain; MG, microwave-irradiated barley grain; SG, steam-flaked barley grain.
[b]ME, metabolizable energy (MJ kg^{-1} DM); SCFA, short chain fatty acid (mMol/200 mg DM); DOM, digestible organic matter (g kg^{-1} DM), A: Potential gas production (mL g^{-1} DM), c: Rate constant of gas production during incubation (mL h^{-1})-(mean ± *SD*); *p*-value = .0001).

TABLE 4 In situ degradability (g kg^{-a} DM, mean ± *SD*) of treated and untreated barley grain [a]

Barley grain	Incubation times (h)							Estimated parameters		
	0	4	8	12	24	36	48	a	b	c
		Ruminal dry matter degradability								
CG	214.1 ± 5.79^{a}	291.7 ± 4.51^{d}	427.2 ± 3.69^{d}	497.1 ± 7.26^{d}	606.9 ± 7.99^{d}	678.2 ± 7.13^{d}	708.5 ± 5.17^{d}	19.13 ± 0.082^{a}	53.86 ± 0.231^{d}	0.065 ± 0.001^{c}
RG	206.3 ± 5.00^{b}	320.6 ± 4.38^{c}	446.8 ± 9.94^{c}	523.5 ± 8.85^{c}	654.4 ± 10.69^{c}	706.4 ± 4.82^{c}	748.0 ± 3.17^{c}	18.66 ± 0.069^{b}	57.33 ± 0.319^{c}	0.073 ± 0.002^{b}
MG	191.8 ± 6.27^{c}	340.8 ± 5.13^{b}	478.3 ± 8.39^{b}	548.5 ± 6.31^{b}	699.8 ± 7.98^{b}	758.6 ± 5.64^{b}	794.9 ± 5.94^{b}	18.33 ± 0.062^{c}	62.55 ± 0.172^{b}	0.073 ± 0.002^{b}
SG	181.7 ± 5.59^{d}	354.9 ± 8.50^{a}	515.4 ± 8.23^{a}	599.2 ± 11.07^{a}	728.5 ± 7.90^{a}	786.6 ± 6.24^{a}	841.7 ± 8.24^{a}	17.35 ± 0.071^{d}	65.95 ± 0.209^{a}	0.084 ± 0.003^{a}
		Ruminal starch degradability								
CG	23.0 ± 1.53^{c}	156.0 ± 2.52^{d}	262.0 ± 2.08^{d}	312.0 ± 2.00^{d}	412.0 ± 1.73^{d}	477.0 ± 1.53^{d}	535.0 ± 2.65^{d}	3.25 ± 0.062^{b}	50.3 ± 0.200^{d}	0.071 ± 0.002^{d}
RG	27.0 ± 1.00^{b}	163.0 ± 2.52^{c}	305.0 ± 3.06^{c}	373.0 ± 2.52^{c}	434.0 ± 2.00^{c}	508.0 ± 2.00^{c}	569.0 ± 1.15^{c}	3.1 ± 0.100^{c}	51.5 ± 0.437^{c}	0.084 ± 0.002^{b}
MG	31.0 ± 1.00^{a}	195.0 ± 2.08^{b}	356.0 ± 2.08^{b}	435.0 ± 2.08^{b}	510.0 ± 2.00^{b}	576.0 ± 2.00^{b}	692.0 ± 2.00^{b}	3.93 ± 0.036^{a}	61.25 ± 0.129^{b}	0.078 ± 0.002^{c}
SG	20.0 ± 1.00^{d}	225.0 ± 1.53^{a}	417.0 ± 1.73^{a}	516.0 ± 0.58^{a}	582.0 ± 1.53^{a}	675.0 ± 1.73^{a}	747.0 ± 1.53^{a}	2.2 ± 0.040^{d}	68.71 ± 0.189^{a}	0.097 ± 0.002^{a}
		Ruminal crude protein degradation								
CG	64.0 ± 3.06^{a}	186.0 ± 3.61 ^{a}	245.0 ± 3.21^{a}	308.0 ± 3.00^{a}	423.0 ± 2.52^{a}	494.0 ± 3.21^{a}	555.0 ± 0.38^{a}	7.26 ± 0.097^{a}	51.78 ± 0.186^{c}	0.046 ± 0.002^{a}
RG	57.0 ± 4.36^{b}	100.0 ± 5.51^{b}	179.0 ± 3.61^{b}	249.0 ± 5.57^{b}	335.0 ± 3.79^{b}	422.0 ± 1.53^{b}	514.0 ± 0.40^{b}	5.5 ± 0.103^{b}	63.22 ± 0.203^{a}	0.023 ± 0.001^{d}
MG	46.0 ± 1.53^{c}	89.0 ± 2.65^{c}	168.0 ± 2.52^{c}	239.0 ± 2.31^{c}	313.0 ± 2.52^{c}	402.0 ± 2.08^{c}	484.0 ± 0.15^{c}	4.35 ± 0.222^{c}	57.63 ± 0.404^{b}	0.029 ± 0.001^{c}
SG	39.0 ± 2.52^{d}	80.0 ± 2.08^{d}	158.0 ± 1.73^{d}	226.0 ± 1.15^{d}	299.0 ± 1.73^{d}	377.0 ± 1.73^{d}	444.0 ± 0.36^{d}	3.5 ± 0.050^{d}	49.88 ± 0.121^{d}	0.035 ± 0.003^{b}

[a–d]Means within a column with different superscripts differ ($p < .05$).

[a]CG, whole barley grain; RG, roasted barley grain; MG, microwave-irradiated barley grain; SG, steam-flaked barley grain, a: soluble fraction, b: slowly degradable fraction, c: fractional disappearance rate constant at which b is degraded (mean ± *SD*); *p*-value = .0001).

different amount of disruption in the protein matrix compared to CG. Partial removal of the protein matrix allowed the starch granules to be digested by rumen microbes. The vastness of starch granules in CG and SG was lower and higher, respectively. The last processing method could embed more colonies of ruminal microorganisms, which could make further holes on the surface. The number of holes increased during incubation, which was the lowest and the highest in CG and SG, respectively.

TABLE 5 Ruminal, post-ruminal and total tract DM disappearance (mean ± *SD*) of treated and untreated barley grain (g kg^{-1} DM)[a]

Barley grain	Ruminal	Post-ruminal	Total tract
CG	497.1 ± 24.09[d]	124.4 ± 23.34[a]	621.5 ± 10.01[d]
RG	523.5 ± 16.36[c]	119.1 ± 12.02[b]	642.7 ± 19.83[c]
MG	548.5 ± 8.14[b]	112.1 ± 23.02[c]	660.6 ± 21.24[b]
SG	599.2 ± 11.55[a]	105.8 ± 10.61[d]	705.1 ± 15.46[a]

[a–d]Means within a column with different superscripts differ ($p < .05$).
[a]CG, whole barley grain; RG, roasted barley grain; MG, microwave-irradiated barley grain; SG, steam-flaked barley grain (mean ± *SD*); *p*-value = .0001).

4 | DISCUSSION

4.1 | Chemical composition and gas production

The DM, ADF, NDF and CP for treated and untreated barley grain differed from that tabulated by NRC (2001) and Zhao et al. (2016). These differences are probably due to the variances in variety, cultivation, processing, fertilisation, species and environmental conditions.

High gas production of cereal grains may be due to their greater concentration of fermentable carbohydrates. Most of the carbohydrates are fermented to short chain fatty acids and gases, especially methane and carbon dioxide (Blümmel and Ørskov, 1993). More than 50% of barley grain carbohydrate is starch; therefore, much gas can be produced from a starch source fermentation of barley grain. There was a significant difference among treatments at 2 hr until 96 hr of incubation in which steam-flaked and control grain had higher and lower ($p < .05$) values than those achieved for the other treatments. The nature of the protein matrix covering starch granules of the most-used cereals in the ruminant diet is more effective than starch content in both rate and extent of digestion (McAllister and Cheng, 1996). In the severe processing methods, such as steam flaking, more fermentation is expected due to the greater starch granule surface with less

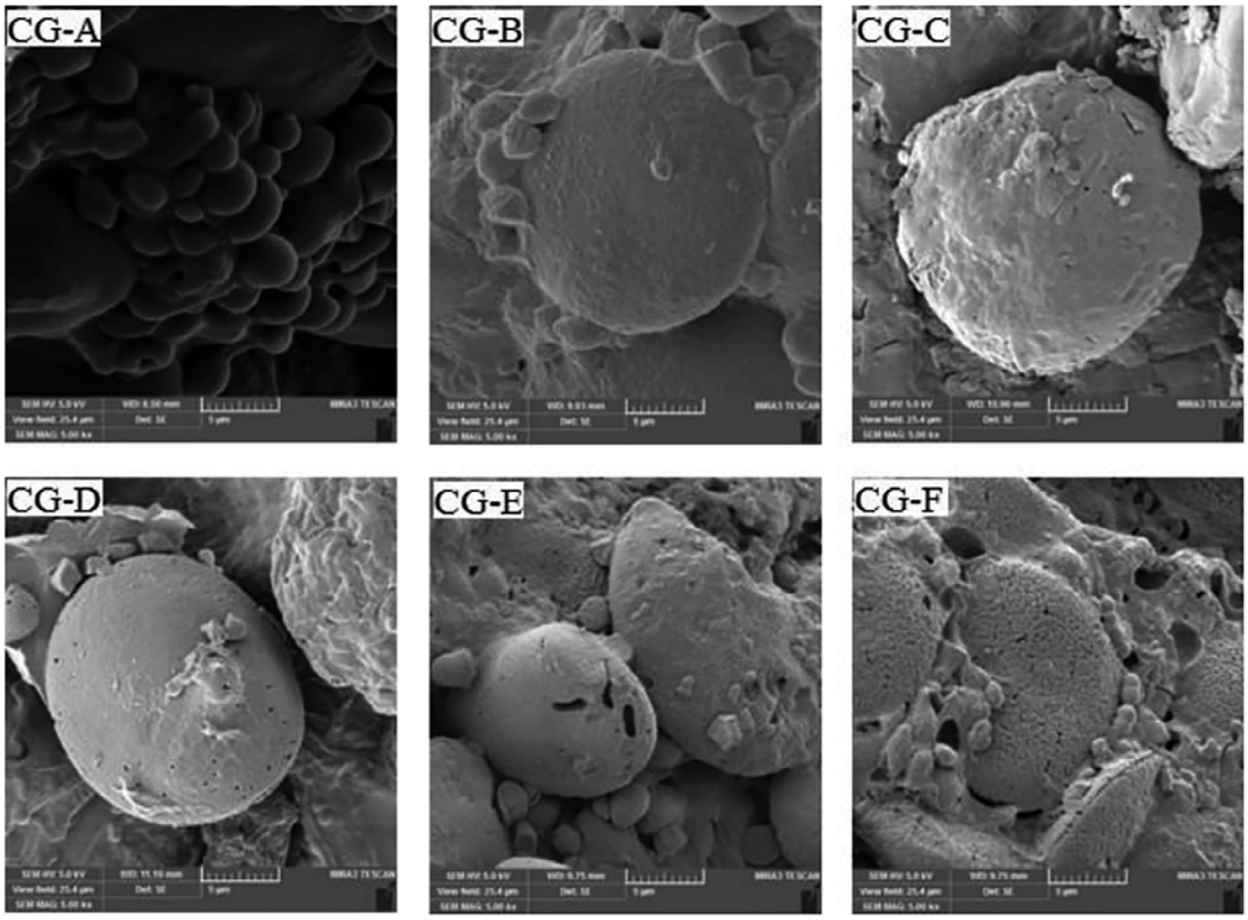

FIGURE 1 FESEM photos (HV = 5.00 KV, MAG = 5.00 KX, Bar = 5.00 μm) for the kinetics of digestion. CG-A to CG-F for 0, 4, 8, 12, 24 and 48 hr of rumen incubation, respectively

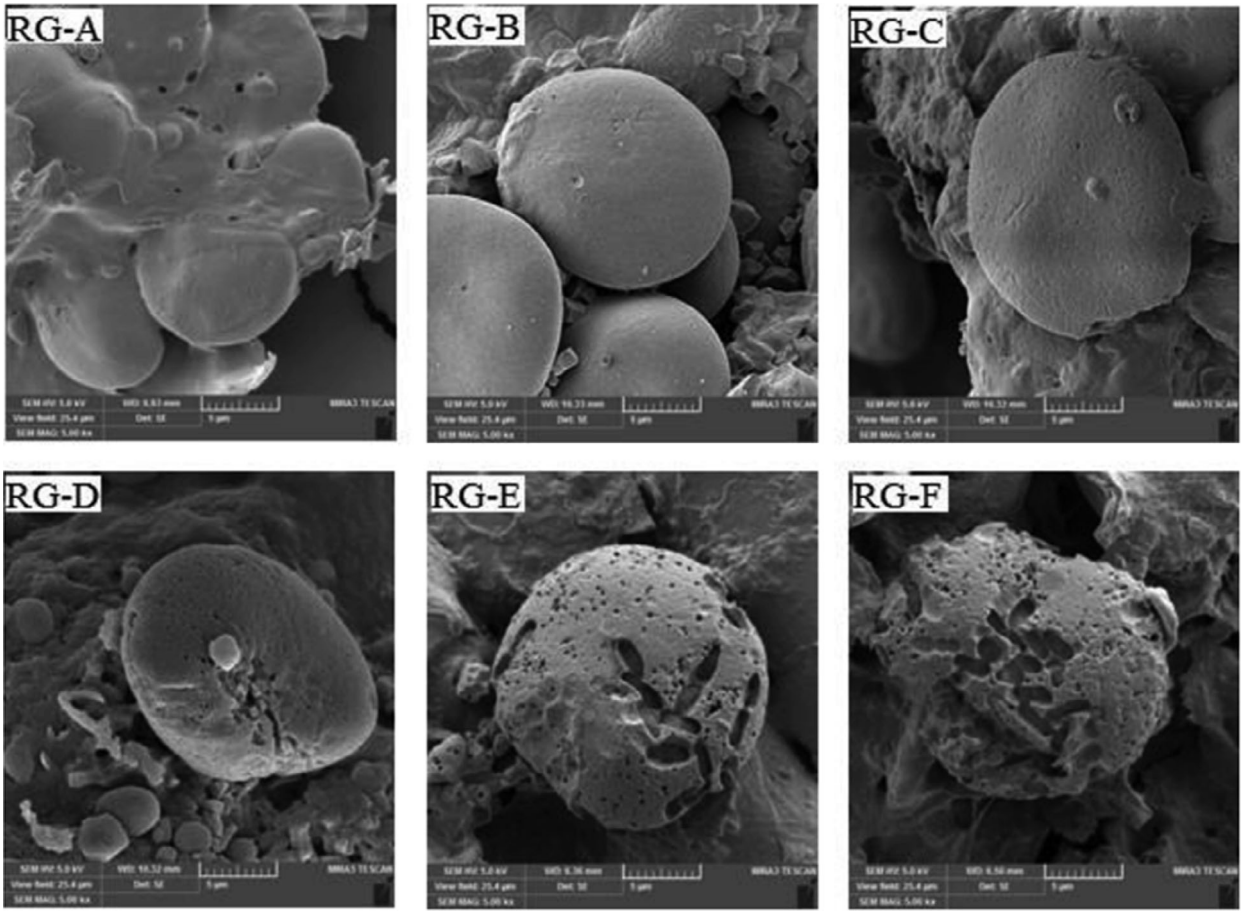

FIGURE 2 FESEM photos (HV = 5.00 KV, MAG = 5.00 KX, Bar = 5.00 μm) for the kinetics of digestion. RG-A to RG-F for 0, 4, 8, 12, 24 and 48 hr of rumen incubation, respectively

protein matrix barrier, which prepares more substrate for rumen biomass, resulting in the reduced effect of the protein barrier, and therefore leading to greater fermentation and gas production. Cumulative gas production data for treated and untreated barley grain agreed with those reported by other researchers (Khajehdizaj et al., 2014; Paya et al., 2014). The estimated higher ME in the SG samples could lead to high potential gas production (*A*) and DOM. An earlier study (Tellez et al. 2006) reported more blood circulation in the total tract because of more SCFA. However, the rate and site of digestion can be important factors for evaluating ruminant performance. Having a greater rate of gas production in SG than in the other treatments can result from increased surface area because of synchronised heat and moisture. Rate of digestion can affect the rumen ecosystem, VFA production and microbial protein synthesis, which can affect animal performance.

4.2 | Ruminal degradability

During ruminal incubation, a decrease in degradability of CG was evident. However, the finer particles will have more losses at zero hour, and in nylon bag technique, it was assumed that starch being washed out of the bags was degraded completely at a rapid rate. Comparing results of nylon bag studies with those of in vivo experiments for starch escaping the rumen revealed an underestimation of the proportion of starch escaping from slowly degraded corn, but an overestimation of rapidly degraded barley, oats and wheat (Nocek and Tamminga, 1991). The SG had the highest starch degradability at the initial 8 hr of incubation versus CG (Table 4). Water absorption and disruption of hydrogen bonds allow accessibility for microbial or enzymatic degradation of the starch granules (Offner et al., 2003). Rumen degradable starch is responsible for only about 60% of the variation associated with the rumen degradable carbohydrate component, revealing that structural carbohydrate digestion can contribute a large portion of the fermentable carbohydrate (Nocek & Tamminga, 1991). Higher degradability is expected to threaten rumen health, especially in high-producing dairy cows that consume greater amounts of grain and are challenged with low rumen pH in the initial time of feeding. Woods, O'Mara, and Moloney (2002) reported different values for starch degradability of CG compared to our data (375.0 vs. 23.0 and 899.0 vs. 535.0 g kg^{-1} DM at 0 hr and 48 hr, respectively). Arieli et al. (1995) showed that heat processing has no effect on starch degradability, whereas Sadeghi and Shawrang (2008) showed that microwave irradiated barley grain for 180 s increased degradability of starch, and more than 300 s reduced extent and rate of starch and CP

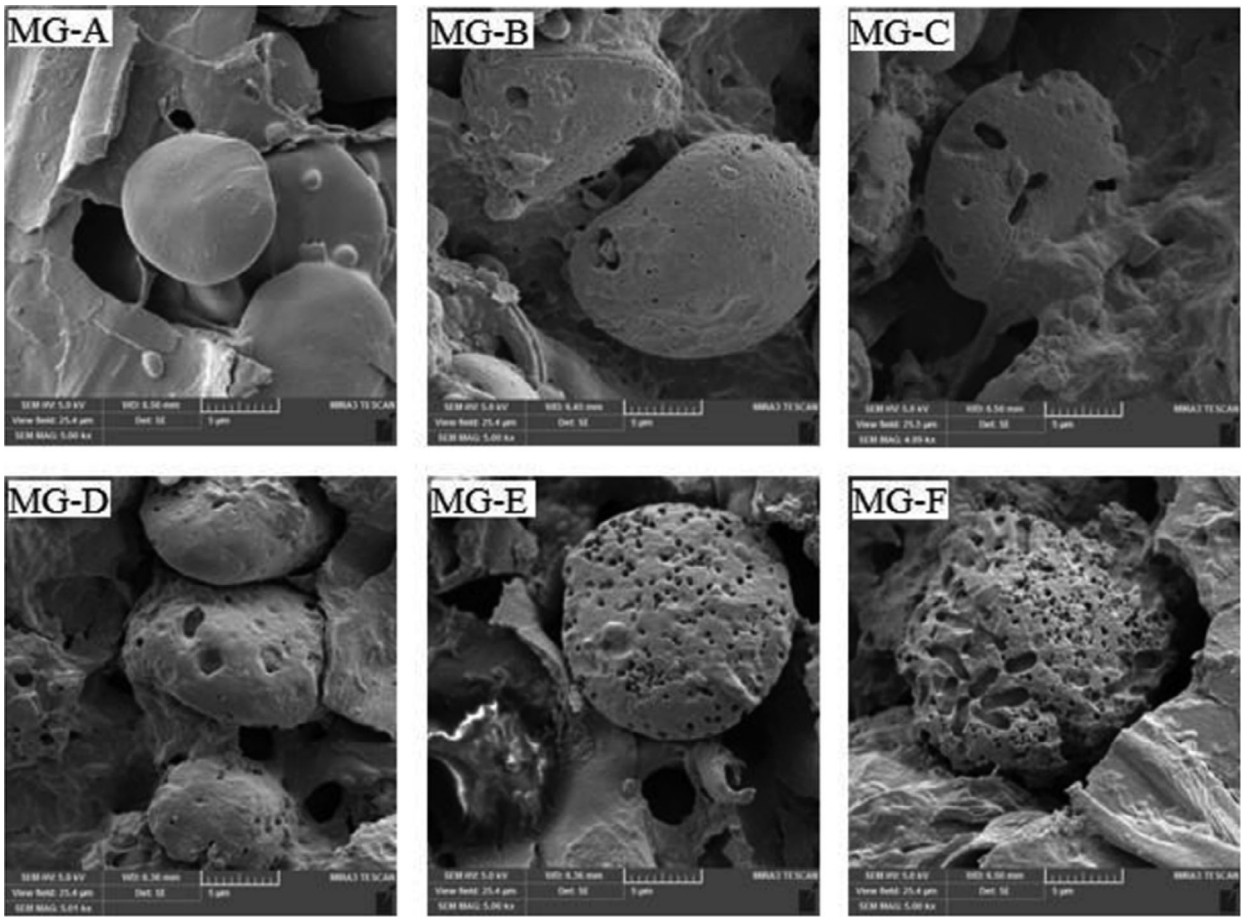

FIGURE 3 FESEM photos (HV = 5.00 KV, MAG = 5.00 KX, Bar = 5.00 μm) for the kinetics of digestion. MG-A to MG-F for 0, 4, 8, 12, 24 and 48 hr of rumen incubation, respectively

degradation. Malcolm and Kiesling (1993) showed that steam flaking of barley grain can increase rumen DM degradability. Ruminal degradability parameters (*a*, *b*, *c*) showed differences ($p < .05$) among the treatments. This finding can be a result of starch gelatinization in treated barley grain, which may reduce the *a* and *c* values, but can increase the *b* value.

4.3 | Intestinal digestion

The results showed that the barley treatments with higher ruminal degradability had lower intestinal digestibility. The disappearance of DM in the intestine was not a result of large intestinal digestibility; other studies also have shown that fermentation in the large intestine has only a restricted effect on the total intestinal disappearance, both in nylon bags (Van Straalen et al., 1997; Voigt et al., 1985) and in in vivo experiments (Van Straalen and Tamminga, 1990). According to the recommendations of Gargallo et al. (2006) and Taghizadeh et al. (2005), pre-incubated samples for 12 hr in the rumen were utilised to determine the intestinal degradability, shows actual rumen retention time is questionable. Incubated cereals for 8 hr in the rumen can reflect the rumen residence time (De Boer et al., 1987). Nonetheless, an 8 hr residence time may be appropriate for cereal grains. In fact, if the ruminal retention time of cereal samples were lower than 12 hr, the actual values of disappearance in the intestine would be higher than those in Table 5. The post-ruminal digestion of RG, MG and SG showed lower values in comparison with CG, indicating that heat-treated barley grain shifted the digestion site. Barley feeding, especially processed barley, because of large ruminant inability to properly chew and break down the husky kernels of whole barley grain, decreases the need for capacious small intestinal absorption, thereby reducing hindgut starch use and faecal nutrient loss absorption (Nikkhah, 2012). Physical or chemical manipulation of barley grain, although it increases VFA, may reduce ruminal pH and cause metabolic disorders, specifically acidosis (Anele et al., 2014; Yang et al., 2013), Therefore, depending on the animal's physiological state, different diet management decisions can be made, considering that increased ruminal degradability of barley grain because of heat treatment can be harmful for rumen health, whereas increasing the total tract digestibility of barley grain can be beneficial.

4.4 | Field emission scanning electron microscope

Using new technologies leads us to more precise data, compared with older methods. Field emission scanning electron microscopy (FESEM) has become a perfect technology in most of the biological sciences

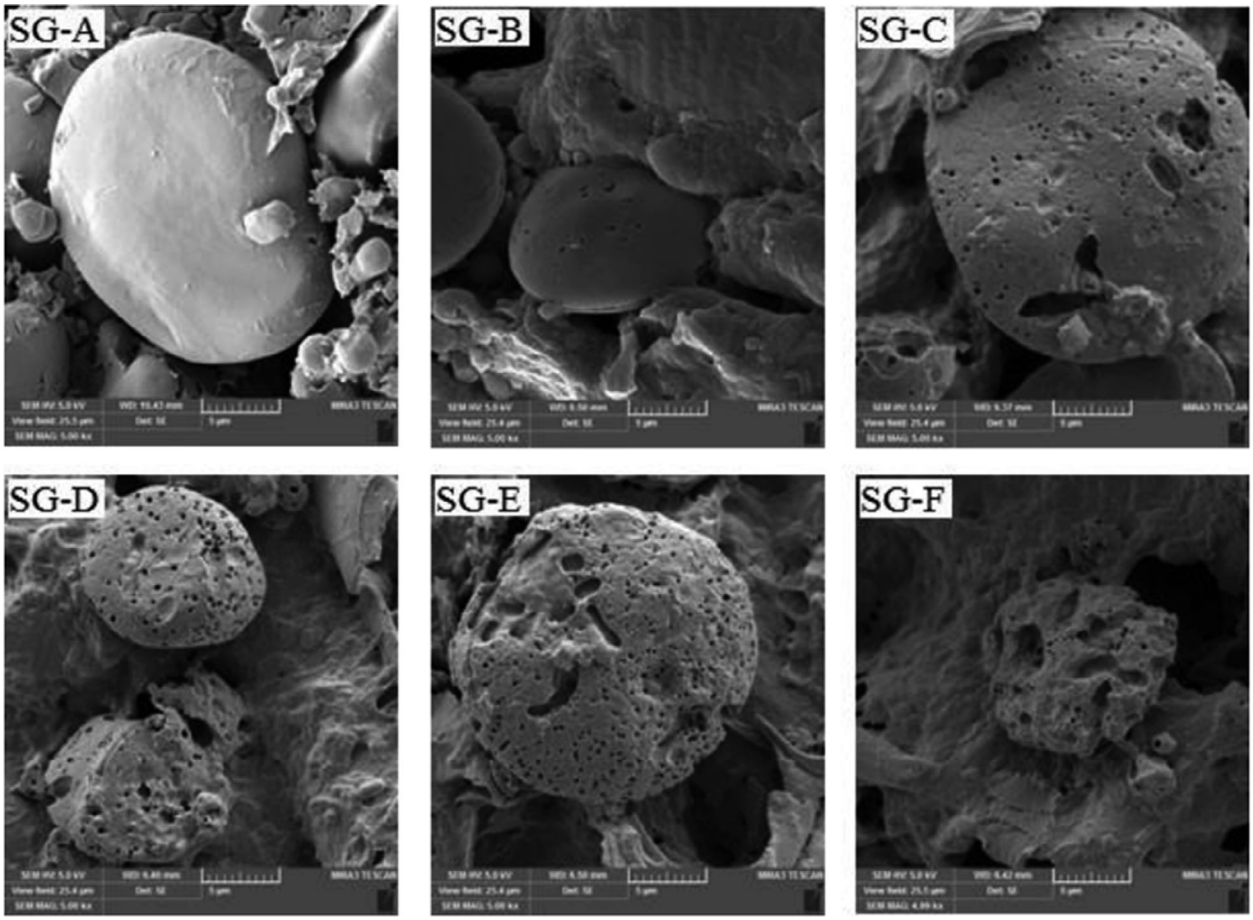

FIGURE 4 FESEM photos (HV = 5.00 KV, MAG = 5.00 KX, Bar = 5.00 μm) for the kinetics of digestion. SG-A to SG-F for 0, 4, 8, 12, 24 and 48 hr of rumen incubation, respectively

and nanotechnology. Advanced FESEM imaging techniques were used broadly in investigating cell morphology, tissue engineering, development of biocompatible materials, and microbiology. Thus, FESEM can offer new insight into ways of enhancing digestibility by having more zoom range and resolution and more depth of field, which aids observation of rugged, perforated, covered surfaces and topography of objects. Limited data are available from images of the contribution of starch to digestibility with special processing. Starch granules are strictly surrounded with a protein matrix. In corn, the granules are within the concentric rings formed during deposition of starch, whereas the protein matrix in barley and wheat is loosely associated with starch granules throughout the entire endosperm (McAllister and Cheng, 1996). Starch granules digestion of barley grain in the rumen starts from a central point of attached microbial colonies on the surface of the granule, resulting in a more accessible surface of the granules, which can increase the digestion rate, although the protein matrix provides a barrier to bacterial attachment. It should be broken down; consequently, we could find holes on the surface of the granules. FESEM photos revealed the effect of the protein matrix covering the granules (Figure 1). Roasting caused shrinkage and disorganisation of the three-dimensional shape of the matrix protein (Figure 2), which could be a reason for reduction in CP degradation of cereal grains. Heat processing caused granules to show up and facilitated bacterial attachment with more colony numbers, resulting in high degradability of starch and DM in barley grain because of high accessibility of fermentable carbohydrates compared to CG. McNiven et al. (1994) found that if the nutrient content of barley is accessed slowly because of roasting, the animal can benefit because of nutrient digestion in the small intestine. Microwave irradiation causes rapid internal heating that evaporates the water inside the grain, and increased pressure ruptures the grain protein coat (Paya et al., 2014). Changes in the physical structure of microwave-treated barley grain can be due to higher pressure and temperature; the starch granules and protein matrix are formed prior to the seed coat (Figure 3). In the present study, we found not only shrinkage but also rupture of the matrix in treated barley. These changes tended toward high ruminal degradability of DM and starch, but low ruminal degradability of CP compared to CG. Yan et al. (2014) reported that microwave irradiation can reduce ruminal degradation of feed CP. They found that microwave irradiation changes protein sub fraction values. The ruminally degradable (PB_1) sub fraction shifts to a partially ruminally degradable (PB_2) sub fraction, which represents the scenario of reduction in ruminal CP degradation rate in the treated barley grains. During the steam-flaking procedure, shrinkage and rupture under different methods occur, while

with flaking, gelatinized granules because of synchronised heat and moisture increase the surface area (Figure 4). The numerous bacterial colonies caused high ruminal degradability of DM and starch for SG. Synchronised heat and moisture not only increase the surface, but they also may cause the least CP degradability. Previous studies (Peng et al., 2014; Yu, 2011) showed that the least CP degradability was associated with a change in protein chemical profile, protein sub fractions, rumen protein degradability, and intestinal digestibility; all are related to changes in protein molecular structure in which the amide I-to-amide II ratio and α-helix-to-β-sheet ratios differ within the treatments.

Different heat processing of barley grain, especially steam flaking increased DM and starch degradability in the rumen resulting in high gas production and high disappearance. Heat-processing decreased ruminal protein degradability and intestinal digestibility of DM and starch. All results were well documented by the number of holes on the starch granule surface and the composition of protein matrix coverage of the starch granule surface using taken images of FESEM. It was concluded that new technologies can be used to describe digestion kinetics and also heat-processing can be used to improve utilisation of barley grain in the ruminants.

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