Gas production and starch degradability of corn and barley meals differing in mean particle size

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ABSTRACT

The objective of this study was to verify the effect of mean particle size (mPS) on both gas production and in vitro rumen starch degradability (IVSD) of corn and barley meals (Cm and Bm, respectively). Batches of the same Cm or Bm were separately processed through 2 different mills (i.e., a cutter mill or a rotor speed mill) equipped with or without different screens to achieve different mPS for each tested meal. Samples were analyzed accordingly to a completely randomized design and the main tested effect of model was mPS ($n = 11$, from 0.46 to 3.50 mm mPS for Cm or $n = 10$, from 0.11 to 2.98 mm mPS for Bm). For both in vitro assays, the rumen inocula were collected from 2 rumen-fistulated Holstein lactating dairy cows fed a total mixed ration with 16.2% crude protein, 28.5% starch, and 35.0% neutral detergent fiber on a dry matter basis. To fit gas production data, 1-pool exponential model and 1-pool or 2-pool Gompertz models were adopted. The rate of gas production decreased and lag increased by increasing mPS of both Cm and Bm, irrespective of adopted 1-pool models. When the 2-pool Gompertz model was used to fit gas production data, a shift of particles from fast to slow fermentable pools was measured by increasing mPS. In particular, the ratio between fast and slow final volumes ranged from 0.90 at 0.11 mm mPS to 0.10 at 2.98 mm mPS for Bm. For Cm, the ratio between fast and slow final volumes decreased quadratically by increasing mPS, with the highest value (i.e., 0.58) measured at the lowest tested mPS. Values lower than 0.10 were measured for mPS greater than 1.93 mm for Cm. Concerning IVSD data, linear decreases in rate of starch degradation equal to -0.049 or -0.092 h^{-1} for each 1-mm increase in mPS were achieved for Cm and Bm, respectively. The 7-h IVSD decreased by 6.3 or 6.5% starch for each 1-mm increase in mPS of Cm or Bm, respectively. Present findings supported the

hypothesis that different particle sizes within the same starch source represent an important factor influencing both fermentation kinetic parameters and IVSD.

Key words: cereal, particle size, gas production, starch degradation

INTRODUCTION

Cereals represent the primary energy source in ruminant diets, being a cost-effective source of digestible energy (Gozho and Mutsvangwa, 2008), and relatively high levels are commonly used in high-producing lactating dairy cow diets to meet their energy requirements (Giuberti et al., 2014). However, endosperm of cereal grains is enclosed by the pericarp, which is extremely resistant to rumen microbial degradation (Huntington, 1997; Dehghan-banadaky et al., 2007). Kernel mechanical processing, such as breaking, cracking, milling, or rolling, makes the starch of grains more accessible to amylolytic digestion by both microorganisms and pancreatic enzyme action (Offner et al., 2003; Hoffman et al., 2012), thus enhancing both rate and extent of starch digestibility in the rumen and in the different tracts of the intestine (Nocek and Tamminga, 1991; Beauchemin et al., 1994; Mathison, 1996). Although processing is essential to maximize the total-tract utilization of starch from grains (Nocek and Tamminga, 1991), kernel over-processing could cause an excessive amount of digested starch in the rumen-reticulum compartment, thus producing great amounts of VFA, a severe pH drop, rumen hypertonicity, lactate production, an excessive flux of propionate to the liver, and rumen disorders (Hindle et al., 2005; Rémond et al., 2004; Gozho and Mutsvangwa, 2008).

Recently, Moharrery et al. (2014) evaluated effects of source, intake, or escape of starch on its digestibility in the 3 major compartments of the digestive tract of lactating dairy cows (i.e., rumen, small intestine, and hind gut) through a meta-analytic approach. As a result, rumen starch digestion was reduced by 1.4% per each kilogram increase in starch intake. Furthermore, rumen starch digestibility lower than 62% starch intake

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for corn and sorghum or greater than 87% starch intake for wheat, barley, oat, or starch from corn silage was reported. However, these authors did not consider the effects of various processing methods as well as variation in mean particle sizes (**mPS**) obtained with different kernel mechanical processing, with these effects being pooled within starch source. Nevertheless, the authors recommended introducing specific corrective coefficients to better model starch digestibility as a function of different mPS, this aspect being recognized as an important factor to predict both site and extent of starch digestion and consequently the nature of the available energy (VFA or glucose) substrates (Firkins et al., 2001; Offner and Sauvant, 2004; Larsen et al., 2009).

Unfortunately, although several *in vivo* studies evaluated lactation performance and starch digestibility for different grain types or various heat processing methods (Joy et al., 1997; Crocker et al., 1998; Tothi et al., 2003), few studies investigated the site and extent of starch digestion as a function of mechanical processing methods. In particular, when these studies were conducted to compare the effect of mechanical processing on performance and nutrient digestibility in lactating dairy cows, only 2 (Yu et al., 1998; Knowlton et al., 1998; San Emeterio et al., 2000) or at most 3 (Callison et al., 2001; Rémond et al., 2004) different mPS levels were tested. To better model the effect of mPS on starch degradation dynamics, a greater number of mPS levels from the same starch source should be used. Such an approach can be favorably carried out by adopting *in situ* or *in vitro* evaluations simulating monogastric (Al-Rabadi et al., 2009, 2012; Bao et al., 2016) or ruminant (Lykos and Varga, 1995; Offner and Sauvant, 2004; Ramos et al., 2009) digestive tracts. Despite *in vivo* studies being the best method to define the dynamics of rumen starch digestion for ruminants (Hindle et al., 2005; Giuberti et al., 2014), the adoption of *in situ* or *in vitro* approaches could represent a good alternative because these methods are characterized by a satisfactory ranking ability (Nocek and Tamminga, 1991; Offner and Sauvant, 2004; Sveinbjörnsson et al., 2007). Furthermore, *in vitro* data provided a good prediction of kinetic dynamics of starch digestion, as declared by Huntington (1997), and this evaluation has also the possibility to be routinely employed in research as well as commercial laboratories. Among *in vitro* methods, a direct measurement of potential rumen starch degradability can be obtained by performing an *in vitro* rumen starch degradability (**IVSD**) test. Usually, the evaluation of IVSD is commonly analyzed after 6 to 8 h of rumen incubations (Cone, 1991; Sveinbjörnsson et al., 2007; Gallo et al., 2014), with 7 h being the most adopted one. This was chosen because it was consid-

ered a reasonable mean retention time of concentrate into the rumen compartment of lactating dairy cows when the method was developed (Allen, 2012). Furthermore, evidence (Krause et al., 2002; Krause and Combs, 2003) indicated the postfeeding times at which the probability of incidence of rumen acidosis increases are close to 7 h. Shorter incubation times (i.e., from 2 to 5 h) have been proposed to improve the ranking ability of IVSD for the evaluation of potential extent of rumen grain degradation (Chai et al., 2004; Giuberti et al., 2014). However, the adoption of shorter incubation times worsened the intra-laboratory method repeatability (Gallo et al., 2016).

The objective of the current study was to model the effect of mPS on gas production kinetic parameters and IVSD of 2 different starch feeds, corn and barley meals (**Cm** and **Bm**, respectively), adopting well-established rumen-based *in vitro* methods. Furthermore, the opportunity to fit gas production data by using 1 or 2 (i.e., fast and slow) digestible pools was evaluated by using different mathematical models.

MATERIALS AND METHODS

Feed Preparation and Chemical Analysis

Three 15-kg batches of dry whole kernel corn (**Cm**) and dry hullless whole kernel barley (**Bm**) were respectively collected over 3 wk (one batch each week) by the same feedstock grains stored in 2 silos located in CERZOO research and experimental dairy farm (San Bonico, PC, Italy) as previously detailed by Gallo et al. (2016). For each sampled batch, subsamples of both Cm and Bm were separately processed following the procedure adopted by Hoffman et al. (2012) through 2 different mills (cutter mill Pulviresette 19; rotor speed mill Pulverisette 14; Fritsch, Idar-Oberstein, Germany) equipped without or with different screens to achieve Cm and Bm with different mPS as reported on Table 1. Then, a representative charge (100 g) of the obtained grounds was run for 10 min through a sieve shaker (Multidimensional Sieveshaker IG/1/S, Giuliani Tecnologie s.r.l., Torino, Italy) equipped with a series of 8 screen sieves with nominal aperture sizes of 4.00, 3.50, 2.50, 1.50, 0.75, 0.50, 0.25, and 0.125 mm, followed by a pan. For each resulting ground, mPS was measured using the ASAE (2003) method S319.3. For both Cm and Bm, a portion of each subsample grounded through a screen above 0.50 mm was re-ground using the rotor speed mill equipped with a 0.5-mm screen and analyzed for total starch content (Megazyme assay kit K-TSTA 07/11; Megazyme International Ireland, Bray, Ireland). Chemical composition of unprocessed Cm and Bm was previously reported (Gallo et al., 2016).

Table 1. Mean particle size (mPS) and particle size distribution (% of total particles) of corn and barley meals processed through 2 different grinding mills equipped without or with different screens¹

Mills ²	Screens (mm)	Transits (no.)	mPS ³ (mm)	Nominal opening of screen sieve (mm)								
				Pan	0.125	0.25	0.50	0.75	1.50	2.50	3.50	4.00
Corn meal												
Rotor speed	0.5	1	0.46 ± 0.04	2.6 ± 0.4	15.0 ± 0.4	26.8 ± 1.0	30.1 ± 1.6	25.6 ± 2.2	—	—	—	—
Rotor speed	1.0	1	0.74 ± 0.02	0.3 ± 0.3	1.2 ± 1.0	5.6 ± 0.7	47.5 ± 1.9	45.5 ± 2.4	—	—	—	—
Cutter mill	1.0	1	0.89 ± 0.02	—	0.4 ± 0.7	2.6 ± 0.6	32.4 ± 1.5	60.5 ± 2.2	4.1 ± 0.8	—	—	—
Cutter mill	2.0	1	1.05 ± 0.09	—	0.2 ± 0.7	0.5 ± 0.4	17.4 ± 3.3	66.8 ± 3.8	15.1 ± 0.2	—	—	—
Cutter mill	3.0	1	1.24 ± 0.01	—	0.1 ± 0.1	1.3 ± 0.1	18.2 ± 1.1	41.5 ± 1.1	32.5 ± 0.7	6.4 ± 0.3	—	—
Cutter mill	4.0	1	1.57 ± 0.02	—	—	1.1 ± 0.2	11.5 ± 1.0	25.9 ± 0.1	43.8 ± 0.7	12.1 ± 1.5	5.7 ± 0.3	—
Cutter mill	None	5	1.93 ± 0.06	—	0.1 ± 0.1	0.5 ± 0.1	4.9 ± 1.2	13.5 ± 2.0	54.2 ± 0.9	18.0 ± 1.0	7.9 ± 1.0	1.0 ± 0.4
Cutter mill	None	4	2.27 ± 0.02	—	0.1 ± 0.1	2.2 ± 0.3	10.1 ± 0.4	11.0 ± 0.1	22.5 ± 0.6	16.0 ± 0.5	24.0 ± 0.4	14.2 ± 0.7
Cutter mill	None	3	2.51 ± 0.09	—	—	1.6 ± 0.2	7.8 ± 2.4	9.6 ± 1.3	15.5 ± 0.3	27.1 ± 3.0	22.4 ± 2.3	16.0 ± 1.4
Cutter mill	None	2	2.94 ± 0.11	—	—	—	5.8 ± 0.7	10.7 ± 1.3	14.4 ± 0.8	12.1 ± 4.2	27.6 ± 4.1	29.4 ± 2.1
Cutter mill	None	1	3.50 ± 0.08	—	—	—	3.1 ± 0.8	7.9 ± 0.9	11.1 ± 1.6	9.6 ± 0.4	24.1 ± 2.9	44.2 ± 6.4
Barley meal												
Rotor speed	0.5	1	0.11 ± 0.01	55.3 ± 2.7	34.7 ± 2.6	6.6 ± 0.9	0.3 ± 0.6	—	—	—	—	—
Rotor speed	1.0	1	0.27 ± 0.08	15.1 ± 0.9	25.1 ± 1.6	27.2 ± 8.0	14.5 ± 1.9	9.1 ± 0.7	—	—	—	—
Cutter mill	1.0	1	0.51 ± 0.01	3.8 ± 0.5	11.8 ± 2.5	19.2 ± 0.9	37.0 ± 1.9	28.2 ± 0.7	—	—	—	—
Cutter mill	2.0	1	0.73 ± 0.03	2.5 ± 1.7	6.8 ± 0.6	7.4 ± 0.2	25.9 ± 0.5	50.5 ± 1.7	6.9 ± 1.3	—	—	—
Cutter mill	3.0	1	1.08 ± 0.02	0.1 ± 0.2	3.6 ± 0.9	5.1 ± 0.1	14.9 ± 0.1	40.0 ± 1.1	36.3 ± 1.3	—	—	—
Cutter mill	4.0	1	1.73 ± 0.02	0.2 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	3.2 ± 0.4	19.8 ± 0.5	60.3 ± 0.3	9.0 ± 0.8	6.2 ± 0.4	—
Cutter mill	5.0	1	2.07 ± 0.02	—	1.0 ± 0.1	1.6 ± 0.1	2.8 ± 0.1	10.1 ± 0.2	42.7 ± 0.8	26.0 ± 0.5	15.8 ± 0.4	—
Rotor speed	None	2	2.31 ± 0.02	—	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.1	8.3 ± 0.6	41.4 ± 0.2	28.3 ± 0.7	20.3 ± 0.7	—
Rotor speed	None	1	2.65 ± 0.02	—	0.3 ± 0.1	0.6 ± 0.1	2.0 ± 0.2	3.0 ± 0.1	26.8 ± 0.5	30.3 ± 0.9	37.0 ± 0.7	—
Cutter mill	None	1	2.98 ± 0.03	—	—	0.5 ± 0.1	1.7 ± 0.3	4.0 ± 0.3	12.7 ± 0.2	20.2 ± 0.9	60.8 ± 1.0	0.1 ± 0.1

¹Values are means ± standard deviations of 3 replicates, 1 for each batch.²Cutter mill Pulviresette 19 and rotor speed mill Pulverisette 14 (Fritsch, Idar-Oberstein, Germany).³Calculated using a sieve shaker (Multidimensional Sieveshaker IG/1/S, Giuliani Tecnologie s.r.l., Torino, Italy) equipped with screen sieves with nominal opening of 4.00, 3.50, 2.50, 1.50, 0.75, 0.50, 0.25, and 0.125 mm, followed by a pan. The mPS of each ground were calculated according to ASAE (2003).

In Vitro Gas Production Test

Samples, one from each batch, were simultaneously incubated in diluted rumen fluid to measure rumen fermentability as gas production according to Menke and Steingass (1988). Two runs were carried out. Briefly, about 220 mg of each sample were weighed into graduated 100-mL glass syringes, then 30 mL of diluted rumen fluid (buffer to rumen ratio of 2:1, vol/vol) collected from the rumen of 2 fistulated lactating dairy cows were added. Rumen liquor was maintained in a warm insulated flask, filtered through 2 layers of cheesecloth, and used within 20 min from collection. Before injection into syringes, the medium was saturated with CO₂ and the pH corrected to 6.5 to 6.6. Donor cows were fed a TMR (16.2% CP, 28.5% starch, and 35.0% NDF on a DM basis) formulated according to the NRC (2001) for an average BW of 600 kg, 140 DIM, and 35 kg milk yield (3.75% fat and 3.35% protein). The bulk of the diet, on a DM basis, was corn silage (31.2%), dehydrated alfalfa hay (16.7%), grass hay (4.1%), and energy-protein supplement (48.0%). Syringes were then placed vertically in a water bath at 39°C. Blanks samples (diluted rumen fluid only) and an internal standard (Gelose 80 maize starch, Penford Food Ingredients Co., Centennial, CO) were incubated. Samples from each batch were considered as experimental replicates. Gas production was measured at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h of incubation.

In Vitro Rumen Starch Degradability

The IVSD was evaluated by an in vitro assay detailed by Sveinbjörnsson et al. (2007) and slightly modified by Gallo et al. (2014). The rumen fluid was collected from the same 2 fistulated dairy cows used for the in vitro gas production test. Samples, one from each batch (i.e., 250 mg of starch for each tested sample), were weighted into 125-mL glass bottles equipped with rubber stoppers. Then, samples were gassed with CO₂ and incubated using 30 mL of the previous described diluted rumen fluid at 39°C in a shaking water bath at 50 rpm. Two runs were carried out. Blanks and an internal standard were also incubated. The IVSD was calculated as the ratio between the amounts of starch disappeared at 7 and 120 h after incubation and the amount of starch in the sample before incubation, after correction for blanks (Sveinbjörnsson et al., 2007). Three bottles were used for each time of rumen incubation. At each experimental time, corresponding bottles were plunged into a bath containing ice to stop fermentation and then residual starch was quantified by adopting a 2-steps enzymatic (i.e., α -amylase and amyloglucosidase hydro-

lysis) approach (Gallo et al., 2014). Samples from each batch were considered as experimental replicates.

Model and Curve Fitting

Three different models were used to fit gas production data and to obtain curve parameters, such as final volume (**Vf**, mL/g of OM), rate of gas production (**kd**, h⁻¹), and lag time (h). In particular, a one-pool exponential model (**EXP**), and 1-pool and 2-pool Gompertz models (**GOM1p** and **GOM2p**, respectively) were adopted. A complete mathematical description of these models has been previously reported by Tedeschi et al. (2008) and Wang et al. (2013). For both models, a discrete lag phase was calculated. A 2-pool exponential model was tested to fit gas production data. Because it did not guarantee a high coefficient of determination and showed a high prediction error, it was not used to fit gas production parameters in the current experiment.

The rate of starch degradation (**kd starch**, h⁻¹) was calculated from IVSD data measured after 7 h of rumen incubation by using a first-order model and assuming a negligible amount of indigestible starch, and was 120 h IVSD values lower than 1 to 2% starch in all analyzed samples (Gallo et al., 2016).

Statistical Analysis

For each meal, gas and IVSD data were evaluated in a complete randomized design by using the MIXED procedure of SAS (version 9.2, 2003, SAS Institute Inc., Cary, NC) with the following model:

$$Y_{ij} = \mu + mPS_i + e_{ij},$$

where Y_{ij} = the response variable, μ = overall mean, mPS_i = fixed effect of mPS ($i = 11$ in Cm and $i = 10$ in Bm), and e_{ij} = random residual error. The linear and quadratic effects of mPS were evaluated by using orthogonal contrasts. The IML procedure of SAS was used to generate contrast coefficients. When significant, the MIXED procedure of SAS was used to obtain regression parameters of linear and quadratic effects of mPS. To study relationships between parameters of interest, the REG procedure of SAS was used. The significance was set at $P < 0.05$.

RESULTS

The mPS of processed cereal meals ranged from 0.46 to 3.50 mm in Cm or from 0.11 to 2.98 mm in Bm (Table 1). The particle size distribution changed by

moving from lowest to highest mPS values for both processed cereal meals. An almost homogenous distribution of particles among different fractions was observed, coefficients of variations being generally lower than 10%. The starch content did not differ among processed meals with average values of 72.2% DM in Cm or 62.5% DM in Bm.

Results from gas production and IVSD tests for Cm are presented on Table 2. When the EXP model was used to fit gas production data, a linear decrease (Figure 1A, $P < 0.05$) in kd was measured by increasing mPS (from 0.062 h⁻¹ at 0.46 mm to 0.038 h⁻¹ at 3.50 mm). Contrarily, the lag quadratically increased ($P < 0.05$) from 0.6 to 2.2 h by increasing mPS. When gas production curve parameters were fitted by the GOM1p model, the kd quadratically decreased ($P < 0.05$) from 0.114 h⁻¹ at 0.46 mm to 0.083 h⁻¹ at 3.50 mm (Figure 1B), whereas lag quadratically increased (from 0.0 h at 0.46 mm to 4.4 h at 3.50 mm, $P < 0.05$) by increasing mPS. Applying a 2-pool model (i.e., GOM2p) to fit gas production data, the kd fast quadratically increased ($P < 0.05$) by increasing mPS (from 0.405 h⁻¹ at 0.46 mm to 1.523 h⁻¹ at 3.50 mm; Figure 1C). The kd slow linearly increased ($P < 0.05$) by increasing mPS, with the lowest (i.e., 0.076 h⁻¹) and highest (0.086 h⁻¹) values measured at 0.89 and 2.27 mm mPS, respectively. A quadratic decrease (Figure 1D, $P < 0.05$) of Vf fast (from 135.9 mL/g of OM at 0.46 mm to 11.9 mL/g of OM at 3.50 mm) was reported when mPS increased in Cm. The Vf slow linearly increased ($P < 0.05$) by increasing mPS, from 234.7 mL/g of OM at 0.46 mm to 342.0 mL/g of OM at 2.94 mm. The ratio Vf fast/Vf slow quadratically decreased (from 0.58 at 0.46 mm to 0.04 at 3.50 mm; Figure 1E, $P < 0.05$) by increasing mPS, whereas lag quadratically increased ($P < 0.05$), ranging from 2.1 h at 0.46 mm mPS to 5.4 h at 2.94 mm mPS. Concerning starch degradation, both 7-h IVSD and kd starch linearly decreased (Figure 1F, $P < 0.05$) by increasing mPS (from 88.5% starch at 0.75 mm mPS to 71.4% starch at 2.94 mm mPS or from 0.322 h⁻¹ at 0.75 mm mPS to 0.180 h⁻¹ at 2.94 mm mPS, respectively).

When the EXP model was used to fit gas production data of Bm (Table 3), a quadratic decrease (Figure 2A, $P < 0.05$) in kd was measured by increasing mPS (from 0.071 h⁻¹ at 0.11 mm mPS to 0.036 h⁻¹ at 2.98 mm mPS). Contrarily, the lag quadratically increased ($P < 0.05$) by increasing mPS (from 0.0 h at 0.11 mm to 1.4 h at 2.98 mm). When gas production curve parameters were fitted by GOM1p model, kd quadratically decreased from 0.159 to 0.070 h⁻¹ (Figure 2B, $P < 0.05$) whereas lag quadratically increased from 0.0 h to 2.1 h ($P < 0.05$) by increasing mPS. A lag equal to 0.0 h was measured for Bm characterized by mPS

from 0.11 mm up to 1.73 mm. Applying the GOM2p model, kd fast (quadratically) and kd slow (linearly) increased by increasing mPS (Figure 2C, $P < 0.05$). A linear decrease of Vf fast (from 170.7 mL/g of OM at 0.11 mm to 29.7 mL/g of OM at 2.98 mm) along with a linear increase of Vf slow (from 190.0 mL/g of OM at 0.11 mm to 313.1 mL/g of OM at 2.65 mm) was measured when mPS increased. The Vf fast/Vf slow ratio quadratically decreased (from 0.90 at 0.11 mm to 0.10 at 2.98 mm; Figure 2E, $P < 0.05$) by increasing mPS, whereas lag linearly increased ($P < 0.05$) ranging from 0.3 to 3.8 h by increasing mPS. The 7-h IVSD and kd starch linearly decreased (Figure 2F, $P < 0.05$) by increasing mPS (from 97.4% starch at 0.11 mm mPS to 72.1% starch at 2.98 mm mPS or from 0.567 h⁻¹ at 0.11 mm mPS to 0.198 h⁻¹ at 2.98 mm mPS, respectively).

DISCUSSION

Rumen starch digestion depends on a dynamic sequence of synergic events influencing fermentation end products and production of carbohydrate sources for postruminal digestion or fermentation. Both rate and extent at which these processes occur in the gastrointestinal tract of ruminants are related to the amount of starch ingested (Allen, 2000; Moharrery et al., 2014) and the type of starch consumed (Nocek and Tamminga, 1991; Offner and Sauvant, 2004). In addition, grain processing, mPS of meals, and most likely passage rate of different particles from rumen-reticulum compartment to small intestine tract are important factors influencing microbial protein synthesis and rumen starch digestibility (Nocek and Tamminga, 1991; Offner and Sauvant, 2004; Ferraretto et al., 2013). In particular, the effect of mPS on starch digestion is related to the available surface area to bacterial attachment or enzymatic degradation (Ferraretto et al., 2013; Giuberti et al., 2014; Gallo et al., 2016), starch digestion being recognized as inversely related to increasing mPS (San Emeterio et al., 2000; Callison et al., 2001; Rémond et al., 2004).

Modern feed evaluation systems such as the NRC (2001), the Nordic feed evaluation system (NorFor, 2011), or the Cornell Net Carbohydrate and Protein Systems (CNCPS; Fox et al., 2004; Higgs et al., 2015; Van Amburgh et al., 2015) take into account the effects of different mPS of meals entering into dairy cow diets. However, correctives proposed by the aforementioned systems are qualitative more than quantitative if a specific digestibility measurement is not available. For instance, the value of truly digestibility for nonfiber carbohydrates of a dry mechanical processed grain should be multiplied by an opportune processing adjustment factor (PAF) depending by particle size of resulting

Table 2. Fitted kinetic parameters of gas production and in vitro rumen starch degradability data of corn meals differing in mean particle size

Gas production data ¹	Mean particle size (mm)											SEM	<i>P</i> <	Orthogonal contrast	
	0.46	0.75	0.89	1.05	1.24	1.57	1.93	2.27	2.51	2.94	3.50			Linear	Quadratic
One-pool exponential model															
Vf (mL/g of OM)	370.8	359.8	369.6	366.9	367.8	366.3	372.3	379.6	375.3	376.9	354.1	6.61	0.312	NS	NS
kd (h ⁻¹)	0.062	0.059	0.056	0.049	0.051	0.044	0.041	0.042	0.042	0.038	0.038	0.0011	<0.05	<0.05	NS
Lag (h)	0.6	0.7	0.7	1.2	1.2	1.6	2.0	1.9	1.9	2.3	2.2	0.06	<0.05	<0.05	<0.05
One-pool Gompertz model															
Vf (mL/g of OM)	356.5	346.4	355.7	351.6	352.8	347.7	350.7	358.9	355.2	353.7	332.8	6.07	0.254	NS	NS
kd (h ⁻¹)	0.114	0.106	0.099	0.091	0.094	0.086	0.086	0.086	0.086	0.083	0.082	0.0022	<0.05	<0.05	<0.05
Lag (h)	0.0	0.0	0.0	1.1	1.0	2.4	3.6	3.3	3.3	4.6	4.4	0.19	<0.05	<0.05	<0.05
Two-pool Gompertz model															
Vf fast (mL/g of OM)	135.9	122.6	121.1	67.0	66.4	36.8	20.7	20.9	25.4	11.9	12.5	3.23	<0.05	<0.05	<0.05
kd fast (h ⁻¹)	0.405	0.404	0.399	0.522	0.495	0.601	0.842	0.999	0.941	1.478	1.523	0.2679	<0.05	<0.05	<0.05
Vf slow (mL/g of OM)	234.7	235.2	245.5	288.9	290.6	313.1	331.0	338.9	331.0	342.0	320.6	7.29	<0.05	<0.05	NS
kd slow (h ⁻¹)	0.078	0.078	0.076	0.083	0.086	0.083	0.085	0.086	0.085	0.084	0.082	0.0016	<0.05	<0.05	NS
Lag (h)	2.1	2.2	2.4	3.2	2.9	3.6	4.5	4.2	4.4	5.4	4.9	0.18	<0.05	<0.05	<0.05
Vf fast/Vf slow (dmnl)	0.58	0.52	0.49	0.23	0.23	0.12	0.06	0.06	0.08	0.03	0.04	0.015	<0.05	<0.05	<0.05
IVSD ²															
7-h IVSD (% starch)	88.1	88.5	86.1	85.9	82.8	79.5	73.6	78.9	72.4	71.4	72.8	2.66	<0.05	<0.05	NS
kd starch (h ⁻¹)	0.307	0.322	0.284	0.288	0.255	0.226	0.194	0.223	0.185	0.180	0.187	0.0229	<0.05	<0.05	NS

¹kd = rate of gas production; Lag = lag time; Vf = final volume. dmnl = dimensionless.
²IVSD = in vitro starch degradation evaluated after 7 h of rumen incubation; kd starch = rate of starch degradation.

Figure 1. Fitted kinetic parameters of gas production (A, curve profile fitted with 1 pool exponential model; B, curve profile fitted with 1-pool Gompertz model; C, D, and E; curve profile fitted with 2-pool Gompertz model) and in vitro starch degradation data (F) of corn meals differing in mean particle size. 7-h IVSD = in vitro starch degradation evaluated after 7 h of rumen incubation; dmsl = dimensionless; kd = rate of gas production; kd starch = rate of starch degradation; Lag = lag time; Vf = final volume. Black or white squares refer to variables of interest displayed on left (primary) or right (secondary) side y-axes, respectively.

meals. In such a case, NRC (2001) provided a PAF only for coarse or ground grains (e.g., dry cracked or ground corn grains with a PAF of 0.95 or 1.00, respectively). The CNCPS suggested a modification of kd of fraction B1 of carbohydrates as a function of kernel grounding. Higgs et al. (2015) stated that kd data are scarce and not routinely estimated for model input. From CNCPS tabular data, specific indications concerning kd of fraction B1 are provided for fine-, medium-, or coarse-ground grains. Last, a correction of passage rate based

on particle length dimension was reported in NorFor (2011), starch feeds with particles length greater or lower than 6 mm being characterized as roughages or concentrates, respectively.

Recently, Hoffman et al. (2012) suggested of introducing a measurement of effective mPS, an adjustment of mPS based on prolamin content of specific grounded Cm, to improve the prediction of fermentation potential of corn grains characterized by different particle distributions. In particular, the mPS effect explained about

Table 3. Fitted kinetic parameters of gas production and in vitro rumen starch degradability data of barley meals differing in mean particle size

Gas production data ¹	Mean particle size (mm)										SEM	<i>P</i> <	Orthogonal contrast	
	0.11	0.27	0.51	0.73	1.08	1.73	2.07	2.31	2.65	2.98			Linear	Quadratic
One-pool exponential model														
Vf (mL/g of OM)	348.2	345.8	342.0	346.5	353.8	356.4	368.2	385.8	371.8	336.3	6.96	0.123	NS	NS
kd (h ⁻¹)	0.071	0.070	0.056	0.051	0.048	0.044	0.039	0.037	0.038	0.036	0.0012	<0.05	<0.05	<0.05
Lag (h)	0.0	0.0	0.2	0.1	0.3	0.7	0.8	0.6	1.0	1.4	0.10	<0.05	<0.05	<0.05
One-pool Gompertz model														
Vf (mL/g of OM)	328.5	326.2	326.3	330.5	338.9	341.5	350.9	368.1	354.7	319.0	6.52	<0.05	<0.05	NS
kd (h ⁻¹)	0.159	0.156	0.107	0.097	0.088	0.079	0.074	0.068	0.071	0.070	0.0035	<0.05	<0.05	<0.05
Lag (h)	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.2	0.9	2.1	0.16	<0.05	<0.05	<0.05
Two-pool Gompertz model														
Vf fast (mL/g of OM)	170.7	134.7	129.4	121.3	108.7	89.2	69.3	60.7	45.0	29.7	4.72	<0.05	<0.05	NS
kd fast (h ⁻¹)	0.337	0.385	0.402	0.374	0.407	0.417	0.403	0.475	0.614	0.761	0.0222	<0.05	<0.05	<0.05
Vf slow (mL/g of OM)	190.0	220.9	218.2	226.6	243.5	261.0	288.3	312.9	313.1	291.4	7.07	<0.05	<0.05	NS
kd slow (h ⁻¹)	0.063	0.066	0.064	0.064	0.065	0.066	0.065	0.063	0.068	0.069	0.0011	<0.05	<0.05	NS
Lag (h)	0.9	0.3	1.8	1.8	2.3	2.7	2.7	2.7	3.1	3.8	0.19	<0.05	<0.05	NS
Vf fast/Vf slow (dmnl)	0.90	0.61	0.59	0.54	0.45	0.34	0.24	0.19	0.14	0.10	0.026	<0.05	<0.05	<0.05
IVSD ²														
7 h IVSD (% starch)	97.4	93.9	93.4	90.2	89.8	87.1	84.9	84.7	82.6	72.1	2.89	<0.05	<0.05	NS
kd starch (h ⁻¹)	0.567	0.439	0.398	0.336	0.326	0.292	0.277	0.278	0.253	0.198	0.0418	<0.05	<0.05	NS

¹kd = rate of gas production; Lag = lag time; Vf = final volume. dmnl = dimensionless.

²IVSD = in vitro starch degradation evaluated after 7 h of rumen incubation; kd starch = rate of starch degradation.

96% of total variability in fermentation potential of a single corn variety with different mPS (i.e., from 0.05 to 3.78 mm). However, when a similar approach was used on a cohort of selected corn grains (i.e., mPS ranging from 0.42 to 2.46 mm), a drastic reduction in explained fermentative profile variance was reported and this was probably imputed to differences in the composition of meals. Several physicochemical properties, including chemical composition, starch granule morphology and crystallinity, endosperm vitreousness, amylose/amylopectin ratio, the presence of lipid-amylose complexes along with protein matrix encapsulating starch granules, can influence starch degradation dynamics as

reviewed by several authors (Tester et al., 2004; Svihus et al., 2005; Lanzas et al., 2007a; Giuberti et al., 2014).

To verify the way by which mPS can influence both in vitro fermentative profile and IVSD data, different batches of the same cereal sources were tested. This permits us to make irrelevant the confounding effect derived from modifications in chemical properties on fermentative and degradability parameters (Al-Rabadi et al., 2012; Hoffman et al., 2012; Gallo et al., 2016). A linear decrease of k_d starch values equal to -0.049 or -0.092 h^{-1} for each 1-mm increase in mPS was measured for both Cm and Bm, respectively. Likewise, a linear reduction in the degree of starch access by

Figure 2. Fitted kinetic parameters of gas production (A, curve profile fitted with 1 pool exponential model; B, curve profile fitted with 1-pool Gompertz model; C, D, and E; curve profile fitted with 2-pool Gompertz model) and in vitro starch degradation data (F) of barley meals differing in mean particle size (mPS). 7-h IVSD = in vitro starch degradation evaluated after 7 h of rumen incubation; dmsl = dimensionless; k_d = rate of gas production; k_d starch = rate of starch degradation; Lag = lag time; VF = final volume. Black or white squares refer to variables of interest displayed on left (primary) or right (secondary) side of y-axes, respectively.

α -amylase of about 27% starch for each 1-mm increase in mPS has been reported in corn samples (Blasel et al., 2006). Furthermore, a linear decrease in the in sacco rumen starch degradability of about 12% starch for each 1-mm increase in mPS of several Cm differing in vitreousness could be estimated by plotting on 2-dimensional graph data reported by Ramos et al. (2009). Similar information were reported by Offner et al. (2003) who referred mPS linearly decreased effective in situ rumen starch degradability by about 16% starch for each 1-mm increase in mPS of several cereal or legume meals. In the current work, the reduction in rumen starch degradability was lower than those reported by aforementioned authors, being 6.3 or 6.5% starch for each 1-mm increase in mPS of Cm or Bm, respectively. Tested meals also differed considering the maximum kd starch values, being 43% greater in Bm than Cm, in agreement with values estimated by Moharrery et al. (2014) for these 2 cereals.

Referring to in vivo data, kd of starch in rumen-reticulum compartment could be estimated as described by Moharrery et al. (2014) using a backward calculation where passage rates, calculated using NorFor (2011) formula, and in vivo rumen starch digestibility values represent the known terms of the equation. Decreases in kd of starch of -0.027 h^{-1} (experiment 2 of Rémond et al., 2004), -0.038 h^{-1} (Knowlton et al., 1998), or -0.070 h^{-1} (San Emeterio et al., 2000) for each 1-mm increase in mPS of Cm could be calculated. Similarly, a linear decrease in kd of starch of -0.019 h^{-1} (experiment 1 of Rémond et al., 2004) or -0.021 h^{-1} (recalculating data from Ferraretto et al., 2013) for each 1-mm increase in mPS of Cm can be estimated. Concerning barley, Beauchemin et al. (2001), feeding steers with ground or coarse Bm, reported a decrease of -0.063 h^{-1} for each 1-mm increase in mPS. Well knowing the hazard of falling into speculative statements resulting from a direct comparison between in vivo versus in vitro data, the entity of the reduction achieved by increasing mPS and estimated by the IVSD approach seems to be comparable with results extrapolated by previously reported in vivo studies.

Furthermore, both rate and extent of rumen fermentation kinetics as a function of mPS were evaluated by carrying out gas production tests. Irrespective of adopted 1-pool models, the kd decreased and lag increased by increasing mPS in both cereals. By passing from the highest to the lowest mPS of Cm, kd reductions by 39 or 28% can be estimated by adopting EXP or GOM1p, respectively. Analogous trends but with greater magnitudes were observed for Bm, with kd reductions equal to about 49 or 57% by fitting gas production data with EXP or GOM1p, respectively. Similarly, Hoffman et al. (2012) adopted a peak absolute rate (**PAR**), the

peak of the first derivative of the smoothed cumulative curves, to represent gas production profile of the same Cm processed through different (i.e., roller, cutter, or cyclone) mills. As reported, PAR ranged from 9.23 mL/0.2 g of DM per h to 2.48 mL/0.2 g of DM per h in mPS of 0.11 or 3.78 mm, respectively. The relationship between PAR as independent variable and mPS as dependent variable was well described by a negative exponential model.

Despite some methodological discrepancies between experiments (e.g., adoption of rumen inoculum employed in fermentation tests, buffer:rumen ratio, type of buffer solution, incubation times, or sample preparation), gas production kinetic parameters could be considered similar to those previously reported for Cm (DePeters et al., 2003; Getachew et al., 2004; Tagliapietra et al., 2010) and Bm (Umucalilar et al., 2002; Anele et al., 2014; Yang et al., 2014). In contrast, Lanzas et al. (2007a), fitting gas production data of 20 barley or 99 corn grain samples collected from a wide range of locations with EXP, reported average rates of gas production 2 to 3 times greater than present findings. Other than different working conditions, the estimated longer lag, 6 h for Cm and 2 h for Bm, as well as the lower asymptotic gas productions, about 335 mL/g of OM for Cm and 290 mL/g of OM for Bm, could contribute to explain discrepancies in kd between experiments.

Foregoing results provide us the opportunity to discuss additional methodological as well as modeling aspects. Referring to different trials carried out to characterize gas production of starch feeds, we noted that different end points were adopted by researchers to model fermentative profile, ranging from 24 h (Anele et al., 2014; Yang et al., 2014) up to 144 h (Tagliapietra et al., 2010). Commonly, the difference in gas production between 2 consecutive incubation times should be lower than 10% of the second to last incubations to clearly identify asymptotic gas production and to obtain an accurate description of gas production profiles (Cone, 1998; Orskov, 2000). In our previous trial (Gallo et al., 2016), we evaluated the gas production of small (i.e., $<0.75 \text{ mm}$) and large (i.e., $>0.75 \text{ mm}$) particles of Cm and Bm by running incubations up to 72 h. When large particles of Cm were fermented, the previous reported recommendation was not respected for all syringes. Therefore, we decided to extend the fermentation up to 120 h considering that some of tested meals had the majority of particles retained on 0.75-mm screen sieve. As a result, the asymptotic gas production was reached after 72 h for finest mPS of Cm and Bm, whereas the use of longer incubation times was required to properly fit gas production profile in Cm with mPS greater than 1.93 mm or Bm with mPS greater than 2.07 mm. Any-

way, adoption of a long-time incubation period could cause a production of gas related to fermentation of difficult to degrade fractions or to the microbial mass turnover (Cone, 1998; Lopez, 2005). To verify this hypothesis, a 3-pool model was run in agreement with mathematical procedure adopted by Cone (1998). Such approach revealed negligible amounts of gas (i.e., lower than 3% of Vf) were produced in this very slowly fermentable pool.

Second, the IVSD represents a direct measurement of starch rumen degradability, whereas the gas production is an indirect one. We carried out the IVSD test by evaluating a single incubation time point. The development of a starch degradability profile by adopting IVSD with a greater number of time incubations appears difficult, with this method being more laborious, time consuming, expensive, and less repeatable than gas production test for this precise scope (Cone, 1991; Giuberti et al., 2014; Gallo et al., 2016). Accordingly, Chai et al. (2004) investigated the possibility to estimate starch degradation kinetics using the gas production technique, starch being fermented simultaneously to other nonsoluble components in starch-rich feeds. Although this study was not specifically designed to test the possible relationship between methods, we verified if measured 7-h IVSD (% starch) could be predicted by gas produced after 7 h of incubation, as estimated by applying the EXP model (GP_{7h} , mL/g of OM). In agreement with Chai et al. (2004), linear relationships were obtained both for Cm [7-h IVSD = $55.18 (SE\ 3.23) + 0.29 (SE\ 0.04) \times GP_{7h}$; $R^2 = 0.87$, $P < 0.01$] and Bm [7-h IVSD = $61.97 (SE\ 4.07) + 0.27 (SE\ 0.04) \times GP_{7h}$; $R^2 = 0.84$, $P < 0.01$], confirming that starch fermentation kinetics could be properly estimated carrying out gas production tests for these kinds of starch feeds.

Last, we noted a tendency in improving the dynamic feed evaluation models by incorporating specific kd for fast or slow digestible pools of carbohydrate fractions. Accordingly to this statement, Zontini et al. (2015) recently presented a feeding trial in which experimental diets were similar in chemical composition but different in the amounts of undigestible NDF or fast and slow digestible NDF pools. Even if data characterizing fast and slow digestible pools of NDF for nonforage fiber feeds and forages are becoming available (Cotanch et al., 2014), to the best of our knowledge information regarding starch feeds is lacking. In the current work, we explored the opportunity to identify fast and slow pools by adopting 2-pool gas production fitting models. Unfortunately, the passage from 1 to 2 pools appears problematic, if not impossible, by using exponential models (Huhtanen et al., 2008; Gallo et al., 2016), with such models showing poor regression performances (low coefficients of determination and high errors of

prediction) and being unable to fit the majority of gas production data for these feeds. For this reason, we adopted a sigmoidal function to test this hypothesis and a shift of both Cm and Bm particles from fast to slow fermentable pools was measured by increasing mPS. Two pools were identified for each tested Bm, whereas the Vf fast/Vf slow ratio in Cm was lower than 0.10 for mPS greater than 1.93 mm. In such a case, the kd fast is more similar to that imputable to soluble carbohydrate and protein fractions more than rapidly fermented starch itself (Lanzas et al., 2007b; Van Amburgh et al., 2015). The foregoing results paid the attention on some drawbacks in the modeling of the 2 pools for these cereals, such as the adoption of a proper model to fit data, optimal incubation times to evaluate the fast and slow pools or factors different from feed sources influencing pool identification. Consequently, future research is warranted to explore the possibility to characterize starch feeds for fast and slow starch digestible pools and to better understand if the characterizations of absolute rates associated with these pools as well as their extent would be important terms for enabling formulation of diets that closely match predicted animal requirements.

CONCLUSIONS

The present findings confirmed the hypothesis that the particle size represents an important factor influencing both fermentation kinetic parameters and in vitro starch digestibility of cereal meals. In particular, both rate and extent of in vitro rumen starch digestibility decreased linearly by increasing mPS of tested Cm and Bm. Therefore, the measurement of the mPS of meals could represent a useful and on-farm applicable tool to propose correctives able to better characterize digestibility dynamics of starch feeds entering dairy cow diets. Concerning modeling perspectives, the passage from 1 to 2 pools intended to improve dynamic feed evaluation models appears problematic when using the exponential model in these kinds of starch feeds and, therefore, other models should be adopted, thus requiring a mathematical adaptation to guarantee a correct estimation of animal requirement and performance. However, the presence of 2 digestible pools should not be retained as a characteristic ascribable to only feed type, but also other factors such as mPS could influence the identification of fast and slow digestible pools within selected starch feeds.

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