A query for effective mean particle size in dry and high-moisture corns

P. C. Hoffman,*¹ D. R. Mertens,† J. Larson,* W. K. Coblenz,‡ and R. D. Shaver*

*Department of Dairy Science, University of Wisconsin, Madison 53706

†Mertens Innovation and Research LLC, Belleville, WI 53508

‡US Dairy Forage Research Center, Marshfield, WI 54449

ABSTRACT

The objective of this study was to investigate whether prediction of fermentation potential (FP) of dry and high-moisture (HM) corns could be improved by using a concept of effective (e) mean particle size (MPS). A set of FP standards was created by processing a single lot of Reid Yellow Dent (RYD) corn to achieve MPS of 3,778, 2,786, 2,282, 1,808, 1,410, 806, 586, 378, 308, 226, and 105 μm . In vitro gas production of RYD standards was measured, and peak absolute rate (PAR) of gas production (mL/0.2 g of DM/h) was used to establish a standard relationship between PAR and MPS. To identify factors other than MPS affecting FP, the MPS and nutrient composition of 36 diverse samples of dry ($n = 18$) and HM ($n = 18$) corns were determined. Composition included dry matter (DM), crude protein, soluble crude protein, neutral detergent fiber, starch, $\text{NH}_3\text{-N}$, prolamin protein, and fat. In vitro gas production of undried, unground dry and HM corns was measured, and PAR, time of peak absolute rate (h), maximum cumulative gas production (mL/0.2 g of DM), gas production fractional rate (h^{-1}), and lag (h) were determined. Nonlinear relationships between MPS, defined as the dependent variable, and PAR, as an independent variable, were used to identify FP deviations unexplained by MPS. When no variation in nutritional composition was present (RYD standards), the relationship between PAR and MPS was described by an exponential decay model [$\text{RYD_MPS} = 9,006 \times e^{(-0.452 \times \text{PAR})}$; $R^2 = 0.96$]. For diverse dry and HM corn populations, the variation in MPS explained by PAR was diminished ($R^2 = 0.50$). To investigate factors that diminish the relationship between MPS and PAR in diverse corns, relative residual (rr) MPS was determined [$\text{rrMPS} = (\text{MPS} - \text{RYD_MPS})/\text{MPS}$], where RYD_MPS was predicted from the PAR of diverse dry and HM corn. The rrMPS was most highly related to prolamin protein [$\text{rrMPS}_{\text{dry}} = 0.58 - 0.15 \times$

(prolamin protein, % of DM); $R^2 = 0.43$] and $\text{NH}_3\text{-N}$ [$\text{rrMPS}_{\text{HM}} = 0.21 + 0.08 \times (\text{NH}_3\text{-N, \% of total N})$; $R^2 = 0.46$] for dry and HM corns, respectively. An eMPS was calculated as $\text{eMPS} = \text{MPS} - \text{MPS} \times \text{rrMPS}$, where rrMPS was predicted from prolamin protein or $\text{NH}_3\text{-N}$ concentration in dry and HM corn, respectively. The natural logarithm of eMPS accounted for 84% of the variability in PAR and 53% of the variability in the fractional rate of gas production. Calculating eMPS by adjusting the MPS of dry corn for prolamin and HM corn for $\text{NH}_3\text{-N}$ concentration improved the assessment of industry corn FP.

Key words: corn, mean particle size, gas production

INTRODUCTION

Dry and high-moisture (HM) corns are typically processed before feeding to enhance their nutritional value for lactating dairy cows. The extent of processing for dry or HM corn is typically measured by measuring mean particle size (MPS; ASABE, 2008), and the effects of MPS on extent and site of digestion of grains by ruminants have been extensively reviewed (Huntington, 1997; Firkins et al., 2001). In vivo, ruminal digestion of corn grains should have an inverse relationship with MPS because the surface area for bacterial digestion or enzymatic hydrolysis of starch increases with smaller MPS (Huntington, 1997). However, this simple inverse relationship between MPS and in vivo starch digestibility is not always observed (Firkins et al., 2001; NRC, 2001). The discrepancy between MPS of corn grains and in vivo starch digestibility occurs when ruminants are fed HM corn. At a common MPS, the digestibility of HM corn is often greater than that of dry corn (Knowlton et al., 1998; Firkins et al., 2001). These observations suggest that relationships between MPS and grain digestibility are different for dry and HM corn and thus make MPS challenging to use in feed grain evaluation systems.

In a recent study, Hoffman et al. (2011) observed physicochemical alterations in HM corn that were related to fermentation during storage. The fate of the starch-protein matrix in HM corn over a long ensiling

Received November 7, 2011.

Accepted January 17, 2012.

¹Corresponding author: pchoffma@wisc.edu

period (240 d) suggested that the MPS of HM corn might not represent the true surface area available to rumen bacteria when degrading HM corn starch granules. Compared with that at 0 d, the α , γ , δ , and β prolamin-zein subunits of the starch-protein matrix at 240 d of ensiling were reduced from 10 to 40%. These observations were verified in electron micrographs (Hoffman et al., 2011), which visually demonstrated that starch-zein protein clusters within particles of HM corn were disassociated and may be less effective in inhibiting bacterial degradation than dry corn with comparable MPS.

Research has also identified negative relationships between endosperm vitreousness in dry corns and in situ starch degradability (Correa et al., 2002; Ngonyamo-Majee et al., 2008). In vivo studies by Allen et al. (2008) and Lopes et al. (2009) demonstrated a negative relationship between vitreousness in dry corn and in vivo starch digestibility. Because starch in vitreous corn endosperm is more extensively encapsulated by prolamin (zein) proteins compared with that in floury or opaque endosperm (Philippeau et al., 2000), vitreous particles of dry corn may be more resistant to bacteria degradation than less vitreous corn particles at similar MPS.

Because the association between starch and proteins within a particle of dry or HM corn may be strong, moderate, or weak, a universal relationship between MPS and grain digestibility has not been incorporated into commercial feed grain evaluation programs. A similar issue occurs in ruminant fiber nutrition because NDF in forage or byproduct feeds can be within short, moderate, or long particles (Armentano and Pereira, 1997). To integrate the effects of NDF concentration and length of the feed particle, physically effective NDF (peNDF) was developed to better define the characteristics of forages and byproduct feeds that meet the minimum fiber requirements for lactating dairy cows (Mertens, 1997). The objective of this study was to identify characteristics of dry and HM corns that measure the inhibition or enhancement of starch fermentation beyond the effects of MPS, and to evaluate a concept of effective MPS (eMPS) that accounts for fermentation potential (FP) differences between and within dry and HM corns.

MATERIALS AND METHODS

FP Standards

A single 50-kg lot of dry whole kernels of Reid Yellow Dent (RYD) corn was chosen as an FP standard because RYD is genetically related to >50% of all corn hybrids grown in the United States (Troyer, 2004). To evaluate

the relationship of MPS on FP, 1,000-g subsamples of RYD corn were processed in a roller mill (Harvestore Systems, DeKalb, IL) with roller clearances set at 4.0, 3.0, 2.0 or 1.0 mm. A second set of 200-g subsamples of RYD corn was processed through a cutter mill (Arthur H. Thomas Co., Philadelphia, PA) fitted with 8.0-, 4.0-, or 2.0-mm screens. A third set of 200-g subsamples was processed through a cyclone mill (Udy Corp., Boulder, CO) fitted with 1.0-, 0.5-, and 0.25-mm screens. After processing, a 100-g subsample of corn processed through the cyclone mill fitted with a 0.25-mm screen was further hand-ground using a mortar and pestle. An additional 100-g sample of pure corn starch (Spectrum Chemical Mfg. Corp., Gardena, CA) was also added as an FP standard. A second set of samples was also added to the FP standards to assess the dynamics of in vitro gas production between the starch and nonstarch fractions in corn. Pure corn starch and 4 samples of distillers grains containing <7.5% starch (Ehrman, 1996) from 4 ethanol production facilities were retained for determination of in vitro gas production. Starch and distillers grains were retained to evaluate gas production potential of the starch and nonstarch fractions of corn, respectively.

The MPS of the various grinds of RYD and of pure corn starch and distillers grain subsamples were measured using methods similar to those of ASABE (2008). Approximately 150 g of processed corn or 100 g of starch was placed on the top of a series of 14 sieves with nominal apertures of 4,750, 3,350, 2,360, 1,700, 1,180, 850, 600, 425, 300, 212, 150, 106, 75, and 53 μ m, followed by a pan. The series of sieves and pan were placed on an oscillating sieve shaker (W. S. Tyler, Mentor, OH) for 0.25 h. The MPS and particle surface area (cm^2/g of DM) were determined by the calculations of Baker and Herrman (2002). The remaining or recovered (pure starch and distillers grains) portion of subsamples was retained for in vitro gas production measurements.

Diverse Corn Populations

Thirty-six dry and HM corns ($n = 18$ each) were selected based on diversity in particle size and nutrient composition from those submitted by the industry to the University of Wisconsin Soil and Forage Analysis Laboratory (Marshfield, WI) for routine analysis. Each sample was split into 3 subsamples, and the first subsample was dried for 48 h at 55°C in a forced-air oven, followed by drying 1 g at 105°C for 3 h to determine DM. The remaining portion of the subsample, dried for 48 h at 55°C, was ground through a cyclone mill fitted with a 1-mm screen and retained for determination of nutrient composition by near infrared reflectance spec-

troscopy (**NIRS**) or chemistry procedures. The second subsample was dried for 48 h at 55°C in a forced-air oven, retained without grinding, and MPS was determined by the method described previously for RYD samples. The third subsample was left undried and unground for determination of *in vitro* gas production.

Laboratory Analysis

The dry and HM corn subsamples that were dried at 55°C and ground through a cyclone mill fitted with a 1-mm screen were packed into cylindrical sample holders equipped with a quartz window and scanned between 400 and 2,498 nm in duplicate using NIRS (model 6500, FOSS-NIR System, Silver Spring, MD) according to the procedures of Marten et al. (1983). The CP, soluble CP (**SOLCP**), NDF, starch, $\text{NH}_3\text{-N}$, and fat concentration of corns were predicted from equations developed by Dairyland Laboratories Inc. (Arcadia, WI). All NIRS calibrations used to predict nutrient composition had high prediction coefficients of determination ($R^2 > 0.90$) and low standard errors of prediction ($< 5.0\%$ of the mean). Prolamin protein was determined on dried subsamples ground through a cyclone mill fitted with a 1-mm screen by using the procedures of Larson and Hoffman (2008).

In Vitro Gas Production

In vitro gas production was measured on RYD FP standards and diverse dry and HM corn populations using the Ankom-RFS system (Ankom Technology Corp., Macedon, NY) fitted with wireless transponders (Leibovich et al., 2009) and using the *in vitro* technique of Goering and Van Soest (1970). *In vitro* gas production measurements were made on all corns in their natural form, meaning samples were not dried or ground before measurement. For RYD FP standards, *in vitro* gas production was measured in a single *in vitro* run on duplicate 0.5-g samples for 36 h in a water bath maintained at 39°C. The FP of pure corn starch and corn distillers grains was also measured in a second *in vitro* gas production run on duplicate 0.5-g mixtures (0.35 g + 0.15 g) of pure corn starch and each of the 4 distillers grains, respectively. Duplicate samples of 0.35 g of starch or 0.15 g of distillers grains were also included in the run. For diverse dry and HM corns, *in vitro* gas production was measured on duplicate 0.5-g samples in 2 *in vitro* runs for a total of 4 gas measurements per sample.

Rumen fluid was obtained from 2 nonlactating dairy cows fitted with ruminal cannulas and fed a diet containing approximately 30% alfalfa-grass silage, 40%

corn silage, and 25% ground dry shelled corn, with the remainder of the diet containing protein, vitamin, and mineral supplements. After collection, rumen fluid was held at 39°C in CO_2 -gassed vessels, mixed in a CO_2 -gassed blender, and strained through 3 layers of cheese-cloth before injection into the gas incubation flask. A blank (rumen fluid only) as well as a standard dry corn ground through a cutter mill (Arthur H. Thomas Co.) fitted with a 4-mm screen were included within each *in vitro* gas production run.

Cumulative *in vitro* gas production pressure of all samples was recorded every 0.25 h, with adjustment for the gas production of a blank sample within run. Gas production values were likewise adjusted for DM content of the sample. Gas production pressure readings were converted to gas volume and expressed as mL/0.2 g of DM, resulting in expression of gas production volumes between 0 and 100 mL (Parissi et al., 2005). For diverse dry and HM corn samples, the difference between the overall average and run average for the standard corn sample was added to each test sample observation to minimize between-run variation.

Calculations and Statistics

For *in vitro* gas production, localized regression (PROC LOESS; SAS Institute, 2001) was used to smooth the cumulative curves and remove measurement noise. Smoothing allowed numerical derivatives to be calculated over 0.25-h intervals without generating erratic results. The LOESS procedure was used with second-degree local polynomial and default options. The LOESS smoothing parameter ranged from 0.1 to 0.2 of total observations.

Peak absolute rate (**PAR**) of gas production, defined as the peak of the first derivative (Lanzas et al., 2007) of the smoothed cumulative curves, was selected to represent the ruminal fermentation potential of the RYD FP standards and diverse dry and HM corns. Numerical derivatives were calculated for each 0.25 h using the smoothed observation before and after the 0.25-h interval. Four additional measures of *in vitro* gas production were used to represent ruminal digestion characteristics of diverse corn population samples. These included time of peak absolute rate (h), maximum cumulative gas production (mL/0.2 g of DM), gas production fractional rate (h^{-1}), and lag time (h). Time of PAR of gas production was determined from derivative curves (Lanzas et al., 2007). Maximum cumulative gas production, gas production fractional rate, and lag time were estimated using a single pool model: $\text{Gas}(t) = G_{\text{max}} \times \{1 - e^{[-k_{\text{s}} \times (t - L)]}\}$, where $\text{Gas}(t)$ is cumulative *in vitro* gas production at time = t , G_{max} is the maxi-

imum cumulative gas production, k_s is the fractional rate of gas production, and L is the discrete lag time. Smoothed cumulative gas production data were fit to the model by PROC NLIN (SAS Institute, 2001) using the Marquardt method with convergeobj = 0.0001, and the grid search for initial parameter estimates for $G_{\max}$ was 70 to 80 by increments of 5. Corresponding initial parameter estimates for k_s and L were 0.06 to 0.24 by increments of 0.09 and 3 to 5 by increments of 1, respectively.

The reference relationship between PAR and MPS for the RYD FP standards was developed using the 2-parameter single exponential decay models ($y = a \times e^{-bx}$) using SigmaPlot v10.0 (Systat Software Inc., Point Richmond, CA). Exponential model parameters from SigmaPlot were confirmed using a natural logarithmic ($\ln$) transformation of the exponential model ($\ln[y] = \ln[A] - b \times [x]$) in the REG procedure (SAS Institute, 2001). The MPS was defined as the dependent variable (y) and PAR as the independent variable (x) so that deviations in MPS unexplained by PAR could be investigated using residual analysis. Relative residual MPS (**rrMPS**) was determined [$\text{rrMPS} = (\text{MPS} - \text{RYD_MPS})/\text{MPS}$] for the diverse population of dry and HM corns, where $\text{RYD_MPS} = 9,006 \times e^{(-0.452 \times \text{PAR})}$; $R^2 = 0.96$, which was the reference relationship for RYD FP standards. Relationships between rrMPS and nutrient composition in dry and HM corn were derived using the first-order polynomial (linear) function in SigmaPlot and separate equations generated the highest R^2 for dry and HM corns. Effective MPS of dry or HMC was calculated as $\text{eMPS} = \text{MPS} - \text{MPS} \times \text{rrMPS}_{\text{dry}}$ or $\text{eMPS} = \text{MPS} - \text{MPS} \times \text{rrMPS}_{\text{HM}}$, respectively, where $\text{rrMPS}_{\text{dry}}$ and rrMPS_{HM} are the predicted rrMPS using separate equations for dry and HM corns, respectively.

SigmaPlot was also used to define the linear relationships between the natural logarithm of eMPS [$\ln(\text{eMPS})$] and PAR or fractional rate of gas production. Cook's Distance Leverage (Cook, 1979) was used to determine if single data points exhibited excessive leverage on regression relationships. Finally, correlations between $\ln(\text{MPS})$ or $\ln(\text{eMPS})$ and PAR, time of PAR, maximum cumulative gas production, gas production fractional rate, and lag times were evaluated using the CORR procedure (SAS Institute, 2001).

RESULTS AND DISCUSSION

FP Standards

The FP standards created by processing a single lot of RYD corn through a roller, cutter, or cyclone mill plus addition of a pure corn starch sample generated

MPS of 3,778, 2,786, 2,282, 1,808, 1,410, 806, 586, 378, 308, 226, 105, and $<53 \mu\text{m}$ (Table 1). The PAR of gas production of RYD FP standards ranged from 2.48 mL/0.2 g of DM/h for the sample with 3,778 μm MPS to 12.27 mL/0.2 g of DM/h for pure corn starch with a MPS $<53 \mu\text{m}$ (Table 1). The relationship between in vitro PAR of gas production and MPS is presented in Figure 1A. The relationship between PAR and MPS was purposely plotted with PAR serving as the independent (x) variable and MPS as the dependent variable (y), which is the inverse of the more conventional presentation where gas production (y) is influenced by MPS (x). Inverse dependent and independent variables were used because the objective of the study was to explore whether differences in MPS effectiveness could be identified using in vitro gas production as a FP surrogate.

The relationship between PAR and MPS of FP standards was described ($R^2 = 0.96$) by a negative exponential model [$\text{MPS} = 9,006 \times e^{(-0.452 \times \text{PAR})}$]. Implications of the relationship between PAR and MPS of RYD FP standards presented in Figure 1A are multifaceted. First, data suggest that when no variation exists in nutrient composition, the relationship between FP (PAR) and MPS is well related. These observations support the mechanism of starch digestion defined by Huntington (1997), in which starch digestion within a single grain source should have an inverse relationship with MPS because the surface area for bacterial digestion or enzymatic hydrolysis of starch increases with smaller MPS. As an extension of this concept, Owens et al. (1986) postulated that differences in starch digestibility between grain types or between fermented and unfermented grains at similar MPS occur primarily due to differences in starch protein matrix bonding, fermentation, or starch type.

An additional implication from the relationship between PAR and MPS (Figure 1A) is that the relationship is exponential and conceptually should be exponential. The exponential relationship is based on the concept that the FP of whole corn kernels ($>5,000 \mu\text{m}$) cannot be <0 , and MPS at the highest fermentation potential cannot be <0 . Theoretically, the relationship between fermentation potential (PAR) and MPS should not cross either the x - or y -axis. However, corn kernels also cannot have an infinitely large size when FP is 0, which explains why PAR crosses the y -axis (MPS) at 9,006 μm (Figure 1A), which is approximately 2-fold greater than the MPS of whole corn kernels. The PAR of pure corn starch (12.3 mL/0.2 g of DM/h) is represented at an MPS of 27 μm .

The dynamic that the total surface area of grain particles is an exponential function of MPS (Baker and

Table 1. Description of fermentation potential standards

Sample	Mill	Screen or clearance, mm	MPS, ¹ μm	Sample weight, g	PAR, ² mL/0.2 g of DM/h
Set 1					
Reid Yellow Dent	Roller	4	3,778	0.50	2.48
Reid Yellow Dent	Roller	3	2,786	0.50	3.12
Reid Yellow Dent	Roller	2	2,282	0.50	3.15
Reid Yellow Dent	Roller	1	1,808	0.50	4.24
Reid Yellow Dent	Cutter	8	1,410	0.50	3.94
Reid Yellow Dent	Cutter	4	806	0.50	5.52
Reid Yellow Dent	Cutter	2	586	0.50	5.29
Reid Yellow Dent	Cyclone	1	378	0.50	5.44
Reid Yellow Dent	Cyclone	0.50	308	0.50	7.73
Reid Yellow Dent	Cyclone	0.25	226	0.50	8.14
Reid Yellow Dent	Cyclone	0.25 + mortar	105	0.50	9.23
Starch	NA ³	NA	<53	0.50	12.27
Set 2					
Starch	NA	NA	<53	0.35	10.00
Distillers grains ⁴	NA	NA	400–550	0.15	0.51
Starch + distillers grains ⁵	NA	NA	81	0.50	10.62

¹MPS = mean particle size.²PAR = peak absolute rate of gas production.³NA = not applicable.⁴Average values for 4 distillers grains sources.⁵In vitro gas production was conducted using a mixture of 0.35 and 0.15 g of starch and distillers grains. The MPS was estimated by difference.

Herrman, 2002) also supports the concept that the relationship between PAR and MPS should be exponential. If FP is related to the exposed surface area of starch, and the surface area of particles is an exponential function of MPS, then PAR should be related to MPS exponentially.

The PAR of isolated pure corn starch (0.35 g), distillers grains (0.15 g), and mixtures of 0.35 g of pure corn starch and 0.15 g of distillers grains are also presented in Table 1. These evaluations were made to mimic corns containing 70% starch with little or no impediment to starch fermentation. The evaluation was conducted to assess the potential effect of starch and nonstarch fractions in corn grain on the study's chosen index (PAR) of FP of corn grain. The average PAR of pure corn starch and distillers grains mixtures was 10.62 mL/0.2 g of DM/h. The PAR of 0.35 g of pure corn starch or 0.15 g of distillers grains when incubated separately was 10.00 and 0.51 mL/0.2 g of DM/h, respectively (Table 1, Figure 1B). The sum of the PAR (10.51 mL/0.2 g of DM/h) for pure starch and distillers grains was similar to that (10.62 mL/0.2 g of DM/h) for the mixtures. The ratio of PAR produced by pure corn starch and distillers grains was approximately 20:1. These data suggest that, when the starch fraction of corn is unimpeded, the nonstarch fraction in corn does not contribute appreciably (~5%) to PAR. Thus, when lesser PAR values are observed, the most likely cause would be a potential impediment of starch digestion.

Diverse Corn Populations

To evaluate the potential influence of nutrient composition on FP beyond that attributed to MPS, diverse populations of dry and HM corns were evaluated. The average MPS of dry and HM corns were 730 and 1,386 μm , respectively, and ranged from 415 to 2,458 μm for dry corns and 300 to 3,906 μm for HM corns (Table 2). Near infrared reflectance spectroscopy was used to predict CP, NDF, starch, SOLCP, $\text{NH}_3\text{-N}$, and fat in dry and HM corns (Table 2) to mimic field application because these nutrients are commonly evaluated using NIRS. For dry corns, the variation of DM, CP, SOLCP, starch, and fat was moderate (<50% of the mean), and dry corn was essentially devoid of $\text{NH}_3\text{-N}$. In contrast, the variation in concentrations of prolamin protein and NDF in dry corns was greater at 116 and 63% of the mean, respectively. The DM content of HM corns ranged from 63.6 to 74.0%. As observed for dry corn, variation of CP and starch in the population of diverse HM corn was minimal (<25% of the mean). In contrast to that in dry corns, concentrations of SOLCP and $\text{NH}_3\text{-N}$ in HM corn were highly variable and exceeded 150% of the mean for $\text{NH}_3\text{-N}$. Variation in prolamin protein, NDF, and fat concentrations in HM corn were similar to that observed for dry corn.

Peak absolute rate of gas production ranged from 3.53 to 8.67 mL/0.2 g of DM/h for dry corns and from 2.17 to 9.51 mL/0.2 g of DM/h for HM corns (Table 2).

Figure 1. (A) The relationship between peak absolute rate (PAR) of gas production and mean particle size (MPS) in corn fermentation potential standards ($R^2 = 0.96$, $\text{SE} = 226$). (B) The PAR gas production potential of pure corn starch (—) and dried distillers grains (---).

Relative variation for the time of PAR of gas production, maximum cumulative gas production, gas production fractional rate, and lag time were similar for dry and HM corns, with most gas production parameters expressing variations exceeding 50% of the mean. Only maximum cumulative gas production of dry corn and lag time of HM corn expressed variations of less than 50% of the mean. Summary statistics of MPS, nutrient composition, and in vitro gas production suggest that dry and HM corn populations were diverse (Table 2).

FP of Diverse Corn Populations

The relationship between PAR and MPS was plotted with PAR as the independent (x) variable, and MPS

as the dependent variable (y), so that residual MPS unexplained by PAR could be calculated (Figure 2). Although PAR accounted for 96% of the variation in MPS of the RYD FP standards [Figure 1A; $\text{MPS} = 9,006 \times e^{(-0.452 \times \text{PAR})}$; $R^2 = 0.96$], PAR accounted for only 50% of the variation in MPS of the diverse populations of dry and HM corn [Figure 2; $\text{MPS} = 4,657 \times e^{(-0.27 \times \text{PAR})}$; $R^2 = 0.50$]. Comparison of the relationships in Figures 1A and 2 provides insight that factors other than MPS affect the FP of dry and HM corns fed to dairy cattle. When nutrient or chemical variation is introduced (Figure 2) due to differing sources of dry corn or fermentation of HM corn or both, the relationship between FP (PAR) and MPS is diminished substantially. One primary reason for the diminished relationship between PAR and MPS in diverse corns compared with RYD FP standards is the recognized difference in FP between dry and HM corn (Knowlton et al., 1998; Philippeau and Michalet-Doreau, 1998). In general, similar PAR was observed when the MPS for HM corn was about 587 μm greater than that for dry corn, consistent with in vivo studies that have observed HM corn starch to be more fermentable than dry corn starch at a similar MPS (Firkins et al., 2001). Secondly, data presented in Figure 2 also suggest greater unexplained variance in the relationship between PAR and MPS within diverse dry corn and HM corn populations than that observed in a single corn (RYD) that varied only in MPS (Figure 1A).

rrMPS and Nutrient Composition

To query nutritional chemistries that could account for variance in the relationship between PAR and MPS within and or between diverse dry and HM corns, rrMPS were calculated in relation to the exponential equation of the RYD FP standards [$\text{RYD_MPS} = 9,006 \times e^{(-0.452 \times \text{PAR})}$]. The rrMPS was calculated because absolute residual MPS can be a function the magnitude of the original MPS (Hendricks, 1931), and we observed better relationships between nutrient composition and rrMPS than for absolute residuals (data not shown). One dry corn residual was removed from the data set because it was determined to be an outlier using Cook's Distance Leverage (Cook, 1979).

Linear slope and intercept estimates are presented in Table 3 for significant correlations ($P < 0.01$) between rrMPS and nutrient compositions of diverse dry and HM corn. For dry corn, a significant correlation was found between prolamin protein ($r = -0.66$) and rrMPS. For HM corn, rrMPS was correlated ($P < 0.01$) with SOLCP (% of DM), SOLCP (% of CP), $\text{NH}_3\text{-N}$ (% of DM), and $\text{NH}_3\text{-N}$ (% of N). For dry corn, rrMPS was best explained by prolamin protein ($\text{rrMPS}_{\text{dry}} =$

Table 2. Summary statistics of mean particle size (MPS), nutrient composition, and in vitro gas production of diverse dry and high-moisture corns

Item ¹	Dry corn				High-moisture corn			
	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
ASABE ² MPS, μm								
>4,750	1.2	0.0	9.0	2.8	12.4	0.5	75.7	20.0
3,350	1.6	0.0	24.4	5.7	11.3	2.0	23.6	6.3
2,360	4.9	0.1	43.3	10.9	16.7	4.2	32.7	7.8
1,700	8.1	0.6	30.2	7.4	13.0	3.0	26.0	5.7
1,180	15.3	4.5	28.0	6.4	9.3	2.7	22.7	4.5
850	17.2	3.0	27.8	6.6	5.6	1.4	10.8	2.2
600	12.7	1.9	26.6	5.0	4.6	1.0	7.6	1.7
425	9.9	1.4	17.0	3.5	4.5	1.3	7.0	1.6
300	6.9	0.5	9.8	2.6	2.6	0.5	4.6	1.1
212	6.7	1.0	15.5	3.0	4.8	1.3	11.3	2.2
150	4.7	0.2	11.2	2.7	1.8	0.4	4.7	1.0
106	7.6	0.5	12.6	4.2	3.3	0.4	10.7	2.4
75	3.6	0.6	7.1	1.6	5.2	1.1	14.0	3.0
53	0.7	0.1	1.5	0.4	4.9	0.8	17.6	4.4
Pan	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0
MPS, μm	730	415	2,458	484	1,386	300	3,906	852
Surface area, cm^2/g of DM	112.3	22.7	161.5	38.2	104.6	17.3	373.0	83.3
Particles, $\times 10^{-4}/\text{g}$ of DM	19.37	0.03	74.73	18.67	83.73	0.04	349.15	19.37
Nutrient								
DM, % as-is	84.7	82.0	89.5	2.1	68.5	63.6	74.0	3.2
CP	9.9	8.8	11.4	0.8	10.2	9.0	11.2	0.7
Soluble CP	2.87	2.34	3.76	0.34	4.73	2.83	6.42	1.06
Soluble CP, % of CP	29.0	22.8	34.0	3.5	46.5	31.4	65.0	10.1
$\text{NH}_3\text{-N}$	0.004	0.000	0.014	0.006	0.060	0.009	0.105	0.026
$\text{NH}_3\text{-N}$, % of total N	0.23	0.00	0.91	0.36	3.66	0.60	6.49	1.61
Prolamin protein	3.33	1.55	5.42	1.19	3.46	2.16	4.61	0.71
NDF	9.6	7.5	13.5	1.4	6.8	5.2	9.1	1.0
Starch	69.4	63.9	72.2	2.4	70.4	67.8	73.9	1.5
Fat	4.08	3.49	5.41	0.46	3.61	2.42	4.29	0.45
In vitro gas production								
Peak absolute rate, $\text{mL}/0.2 \text{ g}$ of DM/h	6.20	3.53	8.67	1.02	6.20	2.17	9.51	1.94
Time of peak absolute rate, h	7.63	5.52	10.00	1.21	6.99	5.42	9.23	0.85
Maximum cumulative gas, $\text{mL}/0.2 \text{ g}$ of DM	77.53	69.52	76.00	8.50	71.32	24.31	83.98	13.22
Gas production fractional rate, h^{-1}	0.12	0.03	0.16	0.03	0.10	0.07	0.16	0.02
Lag, h	4.26	2.80	5.79	0.69	3.81	2.76	4.55	0.41

¹All values are expressed as a percentage of DM unless otherwise defined.

²American Society of Agricultural and Biological Engineers (ASABE, 2008).

0.58 – 0.15 $\times$ prolamin protein; $R^2 = 0.43$); for HM corn, rrMPS was best explained by $\text{NH}_3\text{-N}$ ($\text{rrMPS}_{\text{HM}} = 0.21 + 0.08 \times \text{NH}_3\text{-N}$; $R^2 = 0.46$), when expressed as a percentage of N (Figure 3). Positive rrMPS values in Figure 3 represents MPS that yielded greater PAR than expected, meaning these particles appeared to be less effective at resisting bacterial degradation, or conversely, had a PAR greater than their measured MPS would indicate. Negative rrMPS values in Figure 3 represent MPS that produced lesser PAR than expected, meaning these particles appeared to be more effective at resisting bacterial degradation and had lesser PAR than their measured MPS would indicate.

The observation that prolamin protein was negatively related and $\text{NH}_3\text{-N}$ concentration in HM corn was positively related to rrMPS supports previous findings. Philippeau et al. (2000) observed that vitreous particles of dry corn containing greater amounts of zein proteins

(prolamin) were more resistant to bacterial degradation. Likewise, Hoffman et al. (2011) observed that $\text{NH}_3\text{-N}$ concentration in HM corn was an indicator of fermentation-induced endosperm protein degradation.

Adjusting Measured MPS to eMPS

Because prolamin protein (dry corn) and $\text{NH}_3\text{-N}$ (HM corn) were related to residual differences in MPS, they were used to adjust measured MPS to more effectively estimate the FP of dry and HM corn. As described in the Materials and Methods, $\text{eMPS} = \text{MPS} - \text{MPS} \times \text{rrMPS}_{\text{dry}}$ or $\text{eMPS} = \text{MPS} - \text{MPS} \times \text{rrMPS}_{\text{HM}}$ for dry and HM corns, respectively, where $\text{rrMPS}_{\text{dry}} = 0.58 - 0.15 \times (\text{prolamin protein})$ and $\text{rrMPS}_{\text{HM}} = 0.21 + 0.08 \times (\text{NH}_3\text{-N}, \% \text{ of N})$.

Because $\text{NH}_3\text{-N}$ in dry corn (unfermented) is negligible, $\text{NH}_3\text{-N}$, rather than DM content, was used to

Table 3. Relationships between nutrient composition and relative residuals of mean particle size (MPS) for dry and high-moisture corn¹

Item	Relative residual MPS ²					
	Dry corn			High-moisture corn		
	r	Intercept	Slope	r	Intercept	Slope
DM, % as-is	0.14	—	—	0.16	—	—
CP, % of DM	0.10	—	—	0.21	—	—
Soluble CP, % of DM	0.20	—	—	0.66*	−0.26	0.14
Soluble CP, % of CP	0.06	—	—	0.65*	−0.28	0.02
NH ₃ -N, % of DM	−0.10	—	—	0.67*	0.06	5.95
NH ₃ -N, % of total N	−0.15	—	—	0.68*	0.21	0.08
Prolamin protein, % of DM	−0.66*	0.58	−0.15	−0.06	—	—
NDF, % of DM	0.29	—	—	−0.50	—	—
Starch, % of DM	−0.37	—	—	−0.30	—	—
Fat, % of DM	0.09	—	—	0.02	—	—

¹Slope and intercept values are presented when significant correlations occur between a nutrient and fractional relative residual mean particle size. Relative residual MPS is the difference between measured MPS and MPS predicted (p) for fermentation potential standards [pMPS = 9,006 × e^(−0.452×PAR)], where PAR = peak absolute rate of gas production, expressed as a fraction of the measured MPS.

²Origin MPS determination by the methods of the American Society of Agricultural and Biological Engineers (ASABE, 2008).

*P < 0.01.

classify corns as dry or HM. When NH₃-N was <1.0% of total N, rrMPS_{dry} was used, and for corn samples with NH₃-N >1.0% of total N, the rrMPS_{HM} was used. These equations account for the following in dry and HM corn. When prolamin in dry corn increases, eMPS is increased above the measured MPS to account for the inhibitory effect of prolamin on bacterial starch fermentation (Owens et al., 1986). Conversely, with increasing NH₃-N in HM corn, eMPS is adjusted downward from the measured MPS to adjust for the degradation of the protein matrix in starch granule clusters, which has been demonstrated to enhance starch FP (Hoffman et al., 2011). As a result, calculated eMPS includes the

predominant effect of measured MPS, but also includes the segregated effects of prolamin and NH₃-N on the protein matrix, which have been previously (Owens et al., 1986; Hoffman et al., 2011) demonstrated to alter the FP of dry and HM corn starch, respectively.

The goal of adjusting MPS to eMPS was to generate an index based on MPS and nutritional chemistries that can be used to better predict the FP of corns fed to dairy cattle. Across a population of diverse corn samples, the exponential relationship of PAR with eMPS (Figure 4) explained more of the variation (R² = 0.84 vs. R² = 0.50) than using MPS alone (Figure 2). In addition, the SE of estimation was reduced approximately 3-fold (131 vs. 356) for the relationship between PAR and eMPS compared with PAR and MPS. The improved relationship between PAR and eMPS compared with MPS is observed because MPS alone does not account for nutrient or chemical composition differences in corn, such as degree of starch encapsulation by prolamin-zein proteins (Wallace et al., 1990) or the degree of degradation of starch matrix proteins during fermentation (Hoffman et al., 2011).

Figure 2. Common relationship between peak absolute rate (PAR) of gas production and mean particle size (MPS) for diverse populations of dry (●) and high-moisture (□) corn (R² = 0.50, SE = 356).

Transforming eMPS

Throughout this study, relationships between PAR, MPS, or eMPS were defined with PAR serving as the independent (x) variable and MPS as the dependent variable (y). As previously mentioned, these inverse dependent and independent variables were used because the objective of the study was to find factors that explained the residual variation in MPS associated with nutrient or chemical differences in diverse corn populations. However, in practical applications, eMPS

Figure 3. Relationships between prolamin protein or NH_3-N and fractional relative residual mean particle size (rrMPS) for diverse dry corn (●; $R^2 = 0.43$, $SE = 0.19$) and high-moisture corn (□; $R^2 = 0.46$, $SE = 0.14$), populations, respectively. The fractional rrMPS was determined as $rrMPS = (MPS - pMPS)/(MPS)$. Predicted mean particle size (pMPS) was obtained as $pMPS = 9,006 \times e^{(-0.452 \times PAR)}$, where PAR is peak absolute rate of gas production for each diverse corn. Values for pMPS and MPS are expressed in microns (μm).

would be used to estimate the FP of corn samples. The practical application was used by applying a natural logarithmic transform of eMPS as the independent (x) variable to obtain a linear relationship with FP (e.g., PAR) as dependent (y) variable (Figure 5A). The linear relationship between $\ln(eMPS)$ and PAR is expressed simply as $PAR = 22.9 - 2.64 \times \ln(eMPS)$; $R^2 = 0.84$ (Figure 5). The utility of this transformation would be to estimate or index the FP of dry and HM corns using a simple calculation based on eMPS.

The transforms of eMPS were used to investigate the relationship of eMPS with all in vitro gas production measurements made on diverse dry and HM corn popu-

lations, which included PAR, time of PAR, maximum gas production, gas production fractional rate, and lag time. Correlations with $\ln(MPS)$ or $\ln(eMPS)$ and PAR, time of PAR, maximum gas production, gas production fractional rate, and lag time are presented in Table 4. Compared with $\ln(MPS)$, $\ln(eMPS)$ was better correlated to PAR and time of PAR, and it improved understanding of gas production fractional rate. However, $\ln(eMPS)$ was not more informative in understanding maximum gas production or lag time of diverse dry or HM corns. The relationship between $\ln(eMPS)$ and fractional rate of gas production is presented in Figure 5B, and the relationship is defined as fractional rate $= 0.32 - 0.032 \times \ln(eMPS)$; $R^2 = 0.52$. Similar to the relationship between PAR and $\ln(eMPS)$, estimating the gas production fractional rate from $\ln(eMPS)$ could be used to index the fermentation rate potential of dry and HM corns fed to dairy cattle. We propose that eMPS be determined in future in vitro, in situ, and in vivo experiments that evaluate starch digestion so that its potential utility can be fully evaluated.

Finally, we offer caution in the measurement of MPS of HM corns. During this study, measurement of MPS of dry and HM corns was attempted (data not reported) as-is (undried) with only 5 sieves (4,000, 2,000, 1,000, 500, and 250 μm) and a pan, as might be conducted on-farm. We observed no difference in measuring MPS between the ASABE (2008) and on-farm methods for

Figure 4. Common relationship between peak absolute rate (PAR) of gas production and effective mean particle size (eMPS) of diverse dry (●) and high-moisture (□) corn populations ($R^2 = 0.84$, $SE = 131$). The eMPS of dry or high-moisture (HM) corns was calculated as $eMPS = MPS - MPS \times rrMPS$, where rrMPS is fractional relative residual mean particle size, determined as $rrMPS_{dry} = 0.58 - 0.15 \times \text{prolamin protein}$ and $rrMPS_{HM} = 0.21 + 0.08 \times NH_3-N$, respectively. Values for pMPS and MPS are expressed in microns (μm).

Table 4. Pearson correlations between natural logarithms of mean particle size (MPS), effective MPS (eMPS), and in vitro gas production criteria of diverse corns¹

Gas production criterion	ln(MPS)		ln(eMPS)	
	r	P <	r	P <
Peak absolute rate, mL/0.2 g of DM/h	−0.67	0.001	−0.91	0.001
Time of peak absolute rate, h	0.12	0.95	0.36	0.04
Maximum cumulative gas, mL/0.2 g of DM	−0.48	0.001	−0.45	0.01
Gas production fractional rate, h ^{−1}	−0.67	0.001	−0.73	0.001
Lag, h	−0.09	0.24	0.16	0.35

¹ln(MPS) = natural logarithm of measured MPS; ln(eMPS) = natural logarithm of eMPS.

dry corn, but a significant bias was observed when measuring the MPS of undried HM corn in only 5 sieves. The development of the eMPS concept is based on the measurement of MPS of HM corn dried before sieving,

and we conclude that the eMPS concept is valid only if MPS is measured on predried samples by ASABE (2008) methods.

CONCLUSIONS

The objective of this study was to investigate whether a concept of eMPS in which primary measurement MPS was adjusted for residual factors that affected FP was plausible for diverse industry dry and HM corns. A concept of eMPS is a reasonable index of corn FP if MPS is determined using a standardized methodology and adjusted by routine determinations of prolamin protein and NH₃-N (or SOLCP) for dry and HM corns, respectively. The concept of eMPS provides a measurement that may be useful in predicting animal responses when in vivo validation and or translational models become available.

ACKNOWLEDGMENTS

This project was financially supported by unrestricted gifts from Dairyland Laboratories (Arcadia, WI), Bailey Consulting (Deforest, WI), Vita Plus (Madison, WI), DSM Nutritional Products Inc. (Parsippany, NJ), Rock River Laboratories (Watertown, WI), and Ag-Source Cooperative (Bonduel, WI).

REFERENCES

Allen, M. S., R. A. Longuski, and Y. Ying. 2008. Endosperm type of dry ground corn grain affects ruminal and total tract digestion of starch in lactating dairy cows. *J. Dairy Sci.* 91(E-Suppl. 1):529. (Abstr.)

ASABE (American Society of Agricultural and Biological Engineers). 2008. Methods of determining and expressing fineness of feed materials by sieving. ASAE S319.4. ASABE, St. Joseph, MO.

Armentano, L. E., and M. Pereira. 1997. Measuring the effectiveness of fiber by animal response trials. *J. Dairy Sci.* 80:1416–1425.

Baker, S., and T. Herrman. 2002. Evaluating particle size: MF-2051. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Manhattan.

Cook, R. D. 1979. Influential observations in linear regression. *J. Am. Stat. Assoc.* 74:169–174.

Figure 5. Common relationship between peak absolute rate (PAR) of gas production (A; R² = 0.84, SE = 0.62) or gas production fractional rate (B; R² = 0.53, SE = 0.016) and the natural logarithm of effective mean particle size ln(eMPS) for diverse corn populations.

- Correa, C. E. S., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in situ starch degradability. *J. Dairy Sci.* 85:3008–3012.
- Ehrman, T. 1996. Determination of starch in biomass samples by chemical solubilization and enzymatic digestion. LAP-016. US Department of Energy, National Bioenergy Center, Washington, DC.
- Firkins, J. L., M. L. Eastridge, N. R. St-Pierre, and S. M. Noffsger. 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. *J. Anim. Sci.* 79(E-Suppl.):E218–E238.
- Goering, H. K., and P. J. Van Soest. 1970. Pages 8–11 in *Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications)*. Agric. Handbook No. 379. ARS-USDA, Washington, DC.
- Hendricks, W. A. 1931. The use of the relative residual in the application of the method of least squares. *Ann. Math Stat.* 2:458–478.
- Hoffman, P. C., N. M. Esser, R. D. Shaver, W. Coblenz, M. P. Scott, A. L. Bodnar, R. Schmidt, and B. Charely. 2011. Influence of inoculation and storage time on alteration of the starch-protein matrix in high moisture corn. *J. Dairy Sci.* 94:2465–2474.
- Huntington, G. B. 1997. Starch utilization by ruminants: From basics to the bunk. *J. Anim. Sci.* 75:852–867.
- Knowlton, K. F., B. P. Blenn, and R. A. Erdman. 1998. Performance, ruminal fermentation, and site of starch digestion in early lactation cows fed corn grain harvested and processed differently. *J. Dairy Sci.* 81:1972–1984.
- Lanzas, C., D. G. Fox, and A. N. Pell. 2007. Digestion kinetics of dried cereal grains. *Anim. Feed Sci. Technol.* 136:265–280.
- Larson, J., and P. C. Hoffman. 2008. Technical note: A method to quantify prolamin proteins in corn which are negatively related to starch digestibility in ruminants. *J. Dairy Sci.* 91:4834–4839.
- Leibovich, J., J. T. Vasconcelos, and M. L. Galyean. 2009. Effects of corn processing method in diets containing sorghum wet distillers grain plus solubles on performance and carcass characteristics of finishing beef cattle and on in vitro fermentation of diets. *J. Anim. Sci.* 87:2124–2132.
- Lopes, J. C., R. D. Shaver, P. C. Hoffman, M. S. Akins, and S. J. Bertics. 2009. Type of corn endosperm influences nutrient digestibility in lactating dairy cows. *J. Dairy Sci.* 92:4541–4548.
- Marten, G. C., J. C. Halgerson, and J. H. Cherney. 1983. Quality prediction of small grain forages by near infrared reflectance spectroscopy. *Crop Sci.* 23:94–101.
- Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. *J. Dairy Sci.* 80:1463–1481.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th Rev ed. Natl. Acad. Sci., Washington, DC.
- Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, and J. G. Lauer. 2008. Relationship between kernel vitreousness and dry matter degradability for diverse corn germplasm. II. Ruminal and post-ruminal degradabilities. *Anim. Feed Sci. Technol.* 142:259–274.
- Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. *J. Anim. Sci.* 63:1634–1648.
- Parissi, Z. M., T. G. Papachristou, and A. S. Nastis. 2005. Effect of drying method on estimated value of browse species using and in vitro gas production technique. *Anim. Feed Sci. Technol.* 123–124:119–128.
- Philippeau, C., J. Landry, and B. Michalet-Doreau. 2000. Influence of the protein distribution of maize endosperm on ruminal starch degradability. *J. Sci. Food Agric.* 80:404–408.
- Philippeau, C., and B. Michalet-Doreau. 1998. Influence of genotype and ensiling of corn grain on in situ degradation of starch in the rumen. *J. Dairy Sci.* 81:2178–2184.
- SAS Institute. 2001. *SAS User's Guide: Statistics*. SAS Inst. Inc., Cary, NC.
- Troyer, F. A. 2004. Background of U.S hybrid corn II. Breeding, climate and food. *Crop Sci.* 44:370–380.
- Wallace, J. C., M. A. Lopes, E. Paiva, and B. A. Larkins. 1990. New methods for extraction and quantification of zeins reveal a high content of γ -zein in modified opaque-2 maize. *Plant Physiol.* 92:191–196.