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Influence of single-gene mutations, harvest maturity and sample processing on ruminal *in situ* and post-ruminal *in vitro* dry matter and starch degradability of corn grain by ruminants

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ABSTRACT

Combined effects of single-gene mutations (G), harvest stage (HS) and sample drying technique (DT) on the proportion of dry matter (DM) and starch degraded ruminally *in situ* and post-ruminally *in vitro* were evaluated using four near-isogenic lines in Oh43 inbred background: *floury-2* (*fl2*), *opaque-2* (*o2*), *sugary-2* (*su2*), *waxy-1* (*wx1*) genes and normal Oh43. The inbreds were grown at the University of Wisconsin West Madison Research Station (Madison, WI, USA) during the summer of 2002 in three row plots of 3.04 m × 0.76 m, in a randomized complete block design with three replications. Harvesting was at four stages (HS1 = 1/2 milk-line; HS2 = 5 d post HS1; HS3 = 10 d post HS1; and HS4 = black layer) with samples split for oven drying at 40 °C for 72 h and freeze drying for approximately 60 h. Dried kernels were ground through a Wiley mill (6 mm screen) for measurement of zero hour DM solubility (i.e., A Fraction) and ruminal *in situ* DM degradability (RDMD) after 14 h incubations (1.5 g/bag × 8 replicates in 5 cm × 5 cm bags of 50 μm pore size) using two steers. Residue from the 14 h bags proceeded to an 8 h enzymatic post-ruminal degradation, from

Abbreviations: A Fraction, zero hour solubility or *in situ* bag wash loss; BL, black layer harvesting stage; DM, dry matter; DT, drying technique; FD, freeze drying; G, germplasm or gene mutation type; HS, harvest stage; ML, half milk-line harvesting stage; OD, oven drying; pRDMD, post-ruminal DM degradability; RDMD, ruminal DM degradability; TDMD, total DM degradability; TSTARCHD, total tract starch degradability.

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which the post-ruminal residue was oven dried at 62 °C for 48 h and DM and starch contents determined to provide estimates of total tract DM degradability (TDMD) and total tract starch degradability (TSTARCHD). Three-way interactions for G × HS × DT were observed for the A Fraction (P<0.01) and RDMD (P<0.05). There was compensatory DM degradation post-ruminally from germplasm with low RDMD values (*wx1*(Oh43) and Oh43), thereby reducing inbred differences for TDMD. The influence of advancing maturity on degradabilities was greater for *wx1*, *su2* and Oh43, than for *o2* and *fl2* mutations which have inherently soft endosperm properties. When compared to freeze drying, oven drying at 40 °C reduced the A Fraction, particularly for early harvested samples. The ranking of inbreds for decreasing A Fraction, RDMD and TSTARCHD was *o2*(Oh43) > *fl2*(Oh43) ≥ *su2*(Oh43) > *wx1*(Oh43) ≥ Oh43. Results identify key properties of corn grain germplasm and their relationships with ruminal *in situ* and post-ruminal *in vitro* DM and starch degradability measurements that may be used in advanced corn breeding efforts.

1. Introduction

Corn kernel starch degradability is influenced by numerous recessive mutant genes affecting endosperm properties. These include genes altering starch composition from normal proportions of 0.75 amylopectin and 0.25 amylose (Boyer and Shannon, 2003) to the *waxy* (*wx*) phenotype that is all amylopectin (Watson, 2003). Increased amylopectin makes *wx* corn more susceptible to α-amylase compared to non-*wx* counterparts (MacGregor and Fincher, 1993), thus influencing starch digestibility (Philippeau et al., 1998). The *sugary* (*su*) allele accumulates a water-soluble polysaccharide (WSP) from normal levels of 20–350 g/kg (Marshall and Tracey, 2003). The increase in WSP improved corn kernel starch digestibility in studies by Willcox et al. (1994) that compared starch degradability of a *sugary-Brawn2* (*su-Bn2*) (a close relative of *su2* mutation), with normal dent corn from samples ground using 2 mm Wiley screen. They reported higher A Fraction from *su-Bn2* (301 g/kg) *versus* 143 g/kg from normal dent corn, showing the high potential of *su2* mutation to improve starch degradability. Other mutant genes alter protein composition, with *opaque-2* (*o2*) and *floury-2* (*fl2*) elevating lysine and tryptophan concentrations (Mertz et al., 1964), and *o2* genotypes improved starch utilization by ruminants in several studies (Ladely et al., 1995; Philippeau et al., 1998; Ngonyamo-Majee, 2005).

Besides genetic background, corn starch degradability declined with advancing maturity due to effects of corn vitreousness (Correa et al., 2002), thereby affecting rate, extent and site of starch degradation (Ngonyamo-Majee, 2005). We hypothesized that identifying the extent to which genetic factors influence corn starch utilization, and their interaction with maturity and sample processing conditions, may facilitate advancements in corn breeding programs. This study is unique in that it evaluates germplasm of the same background (Oh43) differing only in single-gene mutations, rather than hybrid comparisons, as in previous studies (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002), to allow better interpretation of genetic effects.

The objectives of this study were to evaluate the combined effects of single-gene mutations, maturity, and sample drying method on the proportion of corn dry matter (DM) and starch degraded ruminally and post-ruminally.

2. Materials and methods

2.1. Corn production

Five isogenic lines of Oh43 inbred background, carrying *fl2*, *o2*, *wx1*, and *su2* mutations were used in this study. The inbred lines were grown at the University of Wisconsin, West Madison Research

Station (latitude 43°05′ North; longitude 89°31′ West and elevation 326 m a.s.l.) during the summer of 2002. The field experimental design was a randomized complete block with three replicates. Plots consisted of three rows of 3.04 m × 0.76 m with planting on 6 May 2002. Stands were thinned to about 10 plants per row before the 6th leaf stage, giving about 60,000 plants/ha. Plots were managed similar to normal corn production practices in surrounding areas. At flowering, each plant was self-pollinated by hand to preserve the endosperm characteristics specific to each inbred. The tasselling and silking time period (i.e., June–July) was dry, so supplementary irrigation was provided to ensure good pollination. However, this early moisture stress may have caused early flowering by all inbreds. At flowering, each plant was self-pollinated by hand to preserve the endosperm characteristics specific to each inbred. The inbreds were harvested at four harvest stages (HS; HS1 = 1/2 milk-line, HS2 = 5 d post HS1, and HS3 = 10 d post HS1 to reflect typical corn silage harvest stages, and HS4 = black layer or about 15 d post HS1 to reflect a typical high-moisture corn grain harvest stage (J.G. Coors, Dept. of Agronomy, University of Wisconsin, unpublished observations). Harvest stages were identified by assessing movement and position of the milk-line on the back side of kernels to result in different harvesting dates for the inbreds. After harvest by hand, ears were frozen in the field within 15 min of harvest to preserve sample quality, and transported to the laboratory. Kernels from the middle portion of ears (2–3 cm from the edges) were shelled when frozen and split in half for oven drying (OD) at 40 °C for 72 h to avoid disruption of kernel cell structure during processing (Philippeau and Michalet-Doreau, 1997) or freeze drying (FD) using Labconco FreeZone 12 L Freeze Dry System Model 77540 (LABCONCO Corp. Kansas City, MO, USA). Samples for FD were initially frozen at −75 °C and later freeze dried over approximately 60 h following manual guidelines (Labconco, 2004).

2.2. Laboratory procedures and degradability measurements

Two steers fitted with ruminal cannulae were fed *ad libitum* a diet composed of 400 g/kg corn grain and 600 g/kg alfalfa hay. Steers were adapted to the diet for 2 weeks prior to the start of ruminal *in situ* incubations. Corn kernels dried as previously described were ground through a 6 mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA) and approximately 1.5 g of the ground material weighed into 5 cm × 5 cm dacron bags with 53 ± 10 μm pore size (Ankom, Fairport, NY, USA) with heat-sealed seams. The *in situ* study was designed to ensure that all treatments were balanced across steers and time. Immediately before being placed in the rumen, bags containing feed were placed in a large mesh bag (36 cm × 42 cm, with a nylon zipper) that was soaked in water (39 °C) for 15 min before insertion into the rumen via the ruminal cannula for 14 h with eight replicates. The choice of rumen incubation time point and sample grind size were based on Sapienza (2002), who reported increased sensitivity of *in situ* rumen assays at 12–16 h incubation times in corn grain degradation with values similar to *in vivo* data in studies conducted at the Livestock Nutrition Center of Pioneer Hi-bred Int. (Johnston, IA, USA). Immediately after removal from the rumen, mesh bags were repeatedly immersed in a 20 L bucket of cold water to remove debris from the outside of the bag and to stop enzymatic activity. Water in the buckets was replaced for the second steer. Mesh bags then were placed in an automatic washing machine filled with water and allowed to agitate on a gentle cycle with the lid open to prevent spinning (Cherney et al., 1990). Bags were then dried at 62 °C for 48 h to determine dry matter content. Zero hour bags were also immersed in water (39 °C) for 15 min before washing to determine DM that is soluble or fine particles that escape through bag pores. After DM content was determined, 14 h bags were subjected to an enzymatic incubation to simulate post-ruminal digestion (Calsamiglia and Stern, 1995). First, bags were incubated in pepsin for 2 h and after rinsing directly incubated in pancreatin buffer for 6 h. The final residue was rinsed and oven dried at 62 °C for 48 h and DM content of the residue was determined and used to calculate total DM degradability (TDMD). Starch content of the residue was also determined using AOAC Method 966.11 (1995) (Woodson-Tenet Laboratories, Des Moines, IA, USA) and total tract starch degradability (TSTARCHD) was calculated. Correa et al. (2002) found the correlation between RDMD and RSTARCHD to be 0.98 ($P<0.0001$) and recommended RDMD as a predictor of RSTARCHD to reduce costs associated with starch analysis of ruminal *in situ* bag residues. Therefore, we analyzed only *in vitro* post-ruminal degradation residues for starch to estimate TSTARCHD. The following formulae were used to calculate proportion of 0 h DM

solubility (i.e., A Fraction), RDMD, post-ruminal DM degradability (pRDMD), TDMD and TSTARCHD:

$$\text{A Fraction (g/kg DM)} = \frac{\text{initial dry weight} - \text{0 h wash residue dry weight}}{\text{initial dry weight}} \times 1000$$

$$\text{RDMD (g/kg DM)} = \frac{\text{initial dry weight} - \text{residual dry weight after 14 h } \textit{in situ} \text{ incubation}}{\text{initial dry weight}} \times 1000$$

$$\text{TDMD (g/kg DM)} = \frac{\text{initial dry weight} - \text{enzyme residue dry weight}}{\text{initial dry weight}} \times 1000$$

$$\text{pRDMD (g/kg)} = \frac{\text{residual dry weight after 14 h } \textit{in situ} \text{ incubation} - \text{enzyme residue dry weight}}{\text{residual dry matter after 14 h } \textit{in situ} \text{ incubation}} \times 1000$$

$$\text{TSTARCHD (g/kg)} = \frac{\text{initial starch weight} - \text{enzyme residue starch weight}}{\text{initial starch weight}} \times 1000$$

2.3. Particle size effects

Mean particle size (MPS) of samples ground through a 6 mm screen for the degradability studies was determined for the inbred lines using dry sieving method #S319.2 according to ASAE (1995). A set of four sieves (4000, 2000, 850, and 350 μm) was used with square apertures and a bottom pan. Material retained on each pan was weighed and the geometric mean particle size was calculated as

$$\text{MPS} = \frac{\log_{i=1}^{-1}\left[\sum W_i \log \bar{d}_i\right]}{\sum_{i=1}^{n} W_i}$$

where d_i = nominal sieve openings of the ith sieve (mm), d_{i+1} = nominal sieve openings in the next larger than ith sieve (mm), $\bar{d}_i = (d_i \times d_{i+1})^{1/2}$, W_i = mass on ith sieve (g), n = number of sieves + 1 (pan).

2.4. Data analysis

The Proc Mixed procedure of SAS (2001) was used to analyze all parameters, with replicate (R; three field plot replicates), and their interaction with corn germplasm (G × R), drying technique (DT × R) and harvest stage (HS × R) as random effects in the model. The effect of steer on RDMD was initially tested and found not significant (i.e., P>0.10), and so it was removed from the model. Tests for relationships between MPS with the A Fraction determined whether there were systematic differences in particle size among the inbreds and whether particle size was linearly related to the A Fraction. Regression analysis showed MPS had a linear relationship with the A Fraction (P<0.001; R^2 = 0.65), and A Fraction values for each inbred were influenced by MPS differently depending on the particle size parameter. As a result, the particle size parameter was used as a covariate (Snedecor and Cochran, 1989; SAS, 2001) to separate effects on degradability that were due to corn genetic background from those that were indirect effects of particle size.

All mean comparisons were by the least significant difference procedure after a significant (P<0.05) treatment effect. Linear regression was used to examine the relationship between TDMD and TSTARCHD using PROC REG of SAS (2001). The two regression lines were statistically analyzed for differences in their slopes and intercepts using ANCOVA (SAS, 2001).

3. Results

3.1. Kernel DM at Harvest

All inbreds had increased DM (P<0.001) with advancing maturity (Table 1). Extent of DM accumulation differed among inbreds (P<0.05) with *fl2*(Oh43) having the highest, and *su2*(Oh43) the lowest values.

3.2. Degradability

Interactions (P<0.05) occurred between G × DT, G × HS, DT × HS and G × DT × HS for the A Fraction and RDMD (Table 1 and Figs. 1 and 2). Total tract DM degradability only had an interaction of G × DT (P<0.05) with a tendency (P<0.10) for DT × HS and G × DT × HS to interact (Fig. 3). Inbreds *o2*(Oh43) and *fl2*(Oh43) had consistently higher (P<0.05) A Fraction than *su2*(Oh43), *wx1*(Oh43) or normal Oh43 with minor variations among maturities for both OD and FD samples. Ruminal DM degradability was consistently higher (P<0.05) for *o2*(Oh43) across HS for both OD and FD treatments. Inbreds *fl2*(Oh43) and *su2*(Oh43) had intermediate performance, with lowest values for *wx1*(Oh43) and normal Oh43. Consis-

Table 1
Comparisons of DM (g/kg), mean particle size (μm) and degradabilities for the different germplasm with advancing maturity for freeze *versus* oven drying methods.

	DM	A Fraction[a]	RDMD[b]	pRDMD[c]	TDMD[d]	GM[e]
Germplasm						
Oh43	617	86	513	611	809	1.088
fl2(Oh43)	646	156	659	518	836	0.966
o2(Oh43)	607	153	715	540	871	0.937
wx1(Oh43)	615	96	528	646	832	1.037
su2(Oh43)	594	108	641	578	850	1.098
Maturity						
HS1[f]	566	148	644	598	862	1.001
HS2[g]	595	124	639	593	856	1.020
HS3[h]	634	106	592	572	829	1.039
HS4[i]	667	100	569	552	811	1.041
Drying method						
Oven drying	631	102	587	579	829	1.062
Freeze drying	600	138	635	579	850	0.989
S.E.	8.8	7.6	19.0	19.1	10.9	0.0116
Statistical significance (P)						
Germplasm (G)	0.050	0.001	0.001	0.001	0.001	0.001
Maturity (HS)	0.001	0.001	0.001	0.001	0.001	0.001
Drying method (DT)	0.001	0.001	0.001	0.001	0.001	0.001
G × DT	NS	0.001	0.010	0.001	0.050	0.001
G × HS	NS	0.010	NS	NS	NS	0.001
HS × DT	NS	0.001	0.010	NS	NS	0.001
G × HS × DT	NS	0.010	0.050	NS	NS	0.001

NS = non-significant.
[a] A Fraction = water-soluble fraction (g/kg DM). See Fig. 1 for interactions.
[b] RDMD = ruminal DM degradability (g/kg DM). See Fig. 2 for interactions.
[c] pRDMD = post-ruminal DM degradability (g/kg DM).
[d] TDMD = total tract DM degradability (g/kg DM). See Fig. 3 for interactions.
[e] GM = geometric mean particle size. See Fig. 5 for interactions.
[f] HS1 = 1/2 milk-line.
[g] HS2 = 5 d post HS1.
[h] HS3 = 10 d post HS1 to reflect typical corn silage harvest stages.
[i] HS4 = black layer or about 15 d post HS1 to reflect a typical high-moisture corn grain harvest stage.

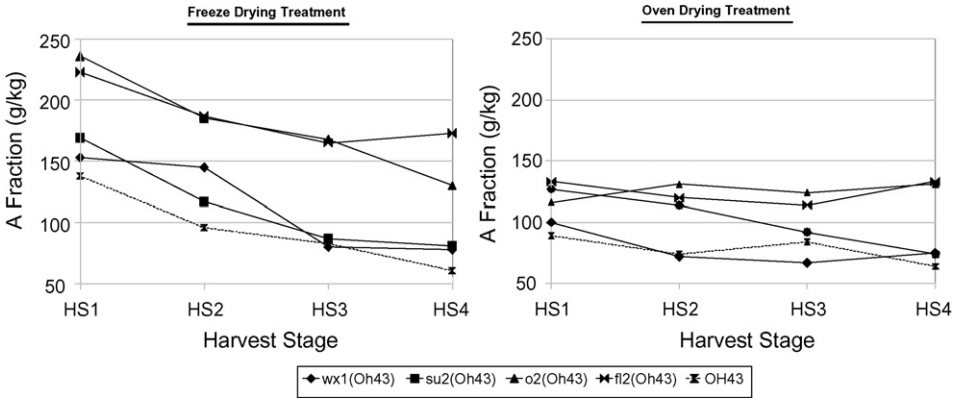

Fig. 1. Interaction of the water-soluble fraction (A Fraction) for the five inbreds with advancing maturity for freeze and oven drying methods [HS1 = 1/2 milk-line; HS2 = 5 d post HS1; and HS3 = 10 d post HS1 to reflect typical corn silage harvest stages; and HS4 = black layer or about 15 d post HS1 to reflect a typical high-moisture corn grain harvest stage]. See Table 1 for statistical significances represented by the differences among lines.

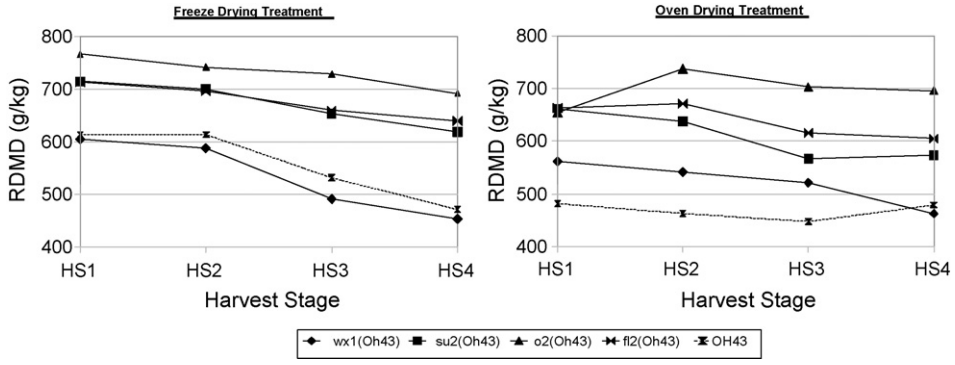

Fig. 2. Interaction of ruminal DM degradability (RDMD) for the five inbreds with advancing maturity for freeze and oven drying methods [HS1 = 1/2 milk-line; HS2 = 5 d post HS1; and HS3 = 10 d post HS1 to reflect typical corn silage harvest stages; and HS4 = black layer or about 15 d post HS1 to reflect a typical high-moisture corn grain harvest stage]. See Table 1 for statistical significances represented by the differences among lines.

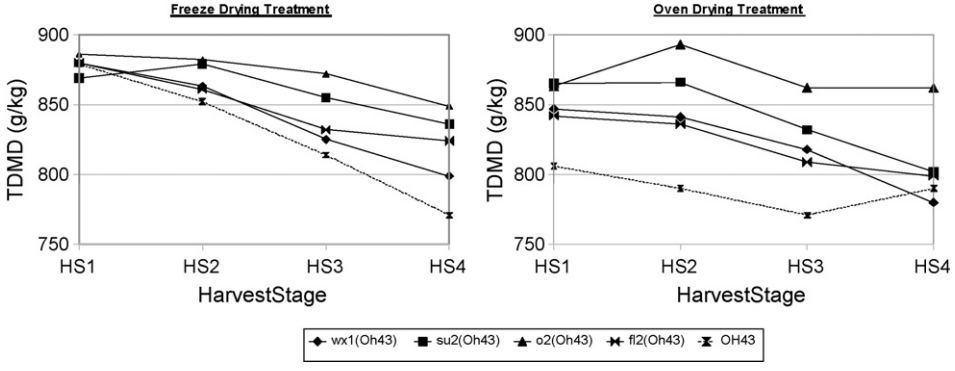

Fig. 3. Interaction of total tract DM degradability (TDMD) for the five inbreds with advancing maturity for freeze and oven drying methods [HS1 = 1/2 milk-line; HS2 = 5 d post HS1; and HS3 = 10 d post HS1 to reflect typical corn silage harvest stages; and HS4 = black layer or about 15 d post HS1 to reflect a typical high-moisture corn grain harvest stage]. See Table 1 for statistical significances represented by the differences among lines.

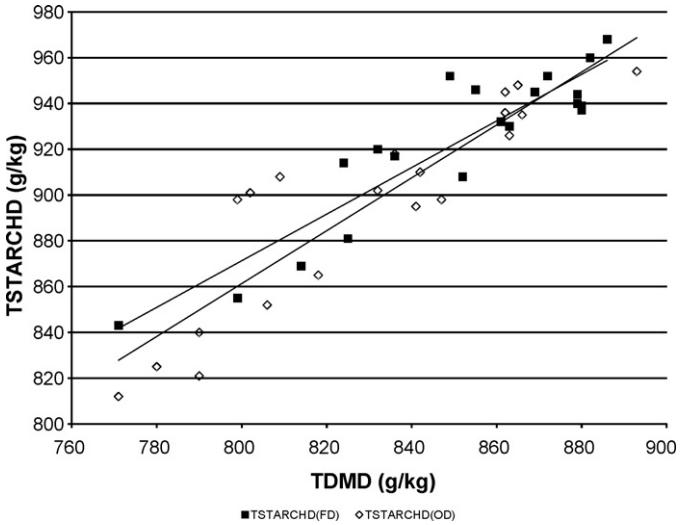

Fig. 4. Regression of total tract starch degradability (TSTARCHD) on total tract DM degradability (TDMD) for freeze drying treatment [TSTARCHD = 1.0181 (TDMD) + 56.812; S.E. = 13.12; R^2 = 0.84; P<0.001] and oven drying treatment [TSTARCHD = 1.1552 (TDMD) − 62.847; S.E. = 15.68; R^2 = 0.80; P<0.001].

tently higher TDMD (P<0.05) was observed for *o2*(Oh43) *versus fl2*(Oh43), *su2*(Oh43) and *wx1*(Oh43), which had intermediate values, or normal Oh43 which was lowest. TSTARCHD had the same inbred differences as for RDMD and TDMD: *o2*(Oh43) > *su2*(Oh43) ≥ *fl2*(Oh43) > *wx1*(Oh43) ≥ normal Oh43. There was a decline in degradability (i.e., A Fraction, RDMD, TDMD) with advancing maturity especially for the *su2*(Oh43) OD treatment, and all five inbreds for FD treatment. Low ruminally degraded *wx1*(Oh43) and normal Oh43 had higher (P<0.05) pRDMD, but lower (P<0.05) pRDMD values occurred for the high ruminally degraded *su2*, *o2* and *fl2*.

3.3. Regressions

Fig. 4 shows the regressions between TDMD and TSTARCHD for FD and OD treatments, which demonstrate strong positive correlations (P<0.001) between TDMD and TSTARCHD of 0.90 and 0.92 for OD and FD, respectively. Comparisons of the two regression lines showed similar slopes (P=0.437) and intercepts (P=0.420).

4. Discussion

4.1. Kernel DM at harvest

The DM content of all inbreds increased with advancing maturity because the kernels increased in size and became more dense (Watson, 2003) as more plant nutrients are partitioned toward the kernel (Jennings et al., 2002a). Watson (2003) reported that the early developmental stage of corn kernels is characterized by accumulation of soluble cellular components, such as soluble N, amino acids, sugars and nucleotides. These components are later metabolized into larger polymers, such as starch and protein, as DM accumulates in the kernel to physiological maturity. Jennings et al. (2002b) found starch granules from mature grain to be larger than those from grain harvested at 1/2 milk-line. The *su2* mutation, which had the lowest DM, was characterized by accumulation of WSP (Marshall and Tracey, 2003) and/or sucrose (Perera et al., 2001) *versus* normal corn. This reduces accumulation of starch in the *su2*(Oh43) kernel endosperm and hence the DM content of the kernel. We suspect that the higher DM for *fl2*(Oh43) resulted from a faster rate of drying in the field rather than increased

starch accumulation *per se*, since only endosperm protein composition is modified with this mutation, as with the *o2*(Oh43). Kernels of floury endosperm were reported by Darrah et al. (2003) to shrink uniformly when drying with very little or no denting, unlike other corn types. This may be a result of limited moisture flow resistance through the modified *fl2* kernel endosperm as the cells are drying.

4.2. Germplasm comparisons for degradability

The A Fraction represents DM that is soluble or fine particle DM that escapes through the *in situ* bag pores. Soft endosperm inbreds *o2*(Oh43) and *fl2*(Oh43) had consistently higher A Fraction DM compared to the hard endosperm inbreds *su2*(Oh43), *wx1*(Oh43) or normal Oh43 across maturities and for both OD and FD treatments. Philippeau and Michalet-Doreau (1997) studied corn hybrids of varying vitreousness ground through a 3 mm screen, and found varying particle size distributions and resultant differences in bag losses for the different hybrids. In our study, we evaluated effects of geometric mean particle size of inbreds ground through a 6 mm screen and used for degradability studies. Data showed a three-way interaction between G × HS × DT ($P<0.001$; Fig. 5 and Table 1), with the more dense and vitreous normal Oh43, *su2*(Oh43), and *wx1*(Oh43) having larger MPS values than *o2* and *fl2* mutations which have softer endosperm. This may explain the larger A Fraction for these soft endosperm mutations, since they had more fine particles that are easily washed out of the bags. However, using MPS as a covariate on A Fraction still maintained germplasm differences with the same three-way interaction ($P<0.05$), an indication that other endosperm properties still influence germplasm differences in A Fraction. These could be related to the 'cementing' properties of the endosperm matrix proteins, limiting solubility of the endosperm constituencies as caused by effects of vitreousness (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002; Ngonyamo-Majee, 2005).

The small differences in the A Fraction between *wx1*, *su2* and normal Oh43 may suggest that some factor other than increased amylopectin proportion in *wx1*(Oh43) or higher sugar content in *su2*(Oh43) influences corn degradability. To ensure that there was no contamination of our *wx1* and *su2* mutations, samples were analyzed for the proportion of amylose in the starch at Truman State University (Kirksville, MO, USA) using the procedure of Williams et al. (1958). Results revealed a proportion of 0.05 amylose in *wx1*(Oh43) and 0.18 amylose in *su2*(Oh43), which agrees with expected values for these mutations (Watson, 2003).

Higher A Fraction appeared to be more related to endosperm protein modifications, as occurs in *o2* and *fl2* mutants that produce soft endosperm characteristics (visual vitreousness rating was 0 for these mutants and their pycnometer density was 1.065 and 1.092 g/cm^3, respectively). The starch

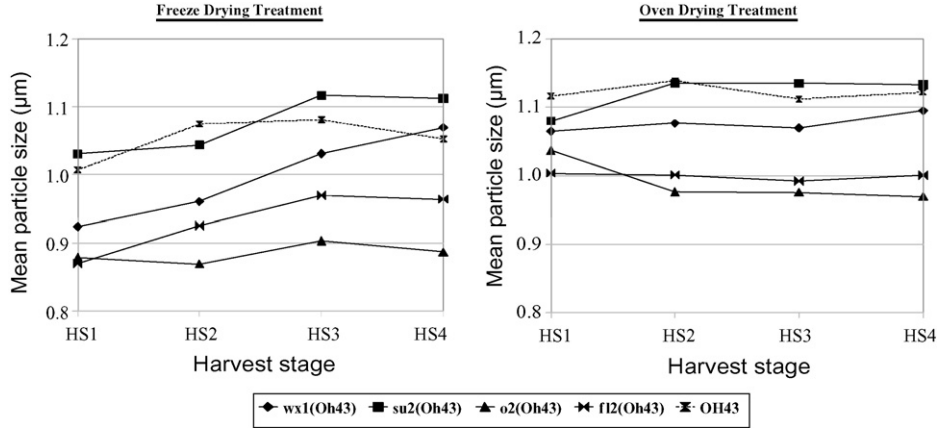

Fig. 5. Mean particle size of the five inbreds at different maturities for freeze and oven drying methods [HS1 = 1/2 milk-line; HS2 = 5 d post HS1; and HS3 = 10 d post HS1 to reflect typical corn silage harvest stages; and HS4 = black layer or about 15 d post HS1 to reflect a typical high-moisture corn grain harvest stage]. See Table 1 for statistical significances represented by the differences among lines.

modification mutations of *su2* and *wx1* had 0.792 and 0.800 vitreousness and 1.225 and 1.200 g/cm^3 density, respectively, which were similar to normal Oh43 values of 0.817 and 1.208 g/cm^3.

4.3. Germplasm comparisons for RDMD and pRDMD

RDMD had consistently higher values from soft endosperm germplasm (*o2*(Oh43) and *fl2*(Oh43)), while the lowest values were in the hard endosperm germplasm (*wx1*(Oh43) and normal Oh43). The *su2*(Oh43) inbred, though hard, had high RDMD values similar to *fl2*(Oh43) across all maturities and for both drying treatments. Correa et al. (2002) found a high correlation ($r = 0.98$; $P<0.001$) between RDMD and RSTARCHD for corn grains, and recommended RDMD as a predictor of RSTARCHD to reduce cost associated with starch analysis of *in situ* bag residues. Therefore, we discuss RDMD results and previous work on RSTARCHD interchangeably. The *fl2* mutation initially generated interest in several breeding programs for its impact on manipulating amino acid composition (Coors and Lauer, 2001), but efforts were slowed because the *o2* genotypes offered more potential since its gene alters the protein matrix surrounding starch granules such that increases in rate and extent of starch degradation occur (Philippeau et al., 1998). This could explain the better performance that we observed from *o2 versus fl2* mutations.

The ability of *su2* mutation to accumulate higher concentrations of sucrose compared to normal corn (Perera et al., 2001) was related to higher degradability values in a study by Willcox et al. (1994), which agrees with our results. Kotarski et al. (1992) showed that waxy corn starch was among the most digestible of all starches, with a protein–starch matrix more easily altered upon exposure to various processing techniques. We were surprised at the lack of marked improvement in ruminal degradability of *wx1* mutation relative to normal germplasm in our study. However, these findings agree with other reports of failure of *wx1* mutation to increase degradability (Philippeau et al., 1998; Schroeder et al., 1998; Michalet-Doreau and Doreau, 1999) or milk yield and composition (Schroeder et al., 1998) over its isogenic normal counterpart.

Our lower RDMD values relative to other studies (Willcox et al., 1994; Philippeau and Michalet-Doreau, 1997; Correa et al., 2002) may be due to the larger particle size, as discussed previously, regarding the A Fraction. Correa et al. (2002) ground samples to pass a 4 mm Wiley screen for their ruminal *in situ* measurements and reported RDMD values as high as 800 g/kg. Our grind size was that recommended by Sapienza (2002) due to RDMD values similar to *in vivo* data. Starch escaping ruminal degradation is partially degraded in the small intestines by amylases with glucose as the end product. Extent of degradability occurring in the small intestines, pRDMD, is dependent on the amount and composition of DM escaping the rumen (Offner and Sauvant, 2004). Therefore, compensatory DM degradation occurs with more DM available for degradation in the small intestines from germplasm with low RDMD values and *vice versa*. This is apparent in the pRDMD data in this study.

4.4. Germplasm comparisons for TDMD and TSTARCHD

The compensatory DM degradation that occurred post-ruminally reduced inbred differences for TDMD. However, germplasm differences on TDMD and TSTARCHD still existed with the same ranking: *o2*(Oh43) > *su2*(Oh43) ≥ *fl2*(Oh43) > *wx1*(Oh43) ≥ normal Oh43. This is not surprising, since corn kernel DM is comprised mostly of starch (750 g/kg; Watson, 2003) which is within the range of 621–768 g/kg starch (DM basis) for our samples across the four maturity stages. Differences between *su2*(Oh43) *versus* normal Oh43 (930 *versus* 861 g/kg) for TSTARCHD were similar to those reported by Willcox et al. (1994) for *su-Bn2 versus* normal dent corn (935 *versus* 897 g/kg, respectively).

4.5. Interactions of corn germplasm, drying method and harvest maturity on degradability

Several interactions occurred between G × DT, G × HS, DT × HS and G × DT × HS for the A Fraction, RDMD and TSTARCHD, but only TDMD had a strong interaction G × DT, and weak interactions for DT × HS and G × DT × HS. This lack of a strong interaction between the three main effects for TDMD was likely related to compensatory degradation occurring post-ruminally as discussed earlier. Evaluation of effects of drying method and harvest maturity on degradability parameters showed increased

(P<0.001) A Fraction, RDMD, TDMD and TSTARCHD values with FD *versus* OD. Philippeau and Michalet-Doreau (1997) found that 40 °C OD reduced *in situ* bag washout relative to FD for corn silage samples. Their *in situ* bag DM washout averaged across grind sizes (2, 4 and 6 mm) were 444 g/kg for FD *versus* 66 g/kg for OD samples. The FD process involves freezing, vacuuming, sublimation and condensing, which could all stress kernels to make them easily shatter and produce more fine particles at grinding.

Although all inbreds were harvested at similar physiological growth stages, it appears they still had different drying rates as shown by their different DM contents and degradabilities at each of the four harvest stages. Paulsen et al. (2003) defined maturity in corn as the cessation of dry weight accumulation by the kernels, or maximum grain yield. Carter and Poneleit (1973) reported that hybrids differ in moisture content at which maximum kernel weight is obtained with moisture contents at BL stage ranging from 150 to 350 g/kg. Kernel endosperm tends to accumulate storage polymers (i.e., starch and protein) with advancing maturity (Jennings et al., 2002b; Paulsen et al., 2003), and become denser (Watson, 2003) and more vitreous (Correa et al., 2002). Therefore, the differences that we observed for degradability parameters across harvest stages could be related to more immature, wetter or less vitreous and less dense kernels crumbling more easily, thereby resulting in higher A Fraction, RDMD, TDMD, and TSTARCHD.

4.6. Regressions

Regression analysis between TDMD and TSTARCHD showed strong relationship between the two degradability parameters at both drying treatments (OD and FD). The strong positive correlations (i.e., 0.90 and 0.92 for OD and FD, respectively), suggest that TDMD can be used to predict TSTARCHD to reduce costs associated with starch analysis on residues from ruminal *in situ* followed by post-ruminal *in vitro* incubations. This agrees with conclusions of Correa et al. (2002) regarding ruminal *in situ* measurements. Results from the comparison of the slopes and intercepts of regression lines show no effect of drying method on the relationship between TDMD and TSTARCHD. This could mean that the increased A Fraction from FD samples discussed earlier was diluted by compensatory degradability in the rumen and post-rumen of both TDMD and TSTARCHD. Hence, sample drying procedure has no impact on total tract degradability parameters in this study. These results also show that TDMD could be used to predict TSTARCHD to reduce cost associated with starch analysis of *in situ* bag residues. The widening gap between the regression lines with declining degradability indicates an increase in the impact of FD treatment on harder endosperm corn. Oven drying samples will therefore produce more consistent degradability values throughout the total digestive tract.

5. Conclusions

Corn starch degradabilities were most affected by the *o2* mutation followed by *su2* and *fl2* mutations which had similar effects. The influence of advancing maturity on all degradability parameters was greater for *wx1* and *su2* mutations and normal corn germplasm (Oh43) than for *o2* and *fl2* mutations which have inherently soft endosperm properties. Freeze drying extracted more moisture from corn grain samples than 40 °C oven drying. However, oven drying at 40 °C for 72 h, or until reaching a stable weight, was more effective for preserving endosperm characteristics in degradability evaluations than did freeze drying. A strong positive correlation between TDMD and TSTARCHD found here, and by other researchers, for ruminal measurements, suggests that DM degradation may provide a low cost and reliable predictor of starch degradability in corn grain.

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