GRIND SIZE AND METHOD AFFECT RUMEN DIGESTIBILITY IN COMMERCIAL ANALYSIS

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AGRICULTURAL ANALYSIS

Objective: To determine if lab rumen digestion methodology affected dry matter (DMD) and starch digestion (SD) across a wide range of sample types.

Materials and Methods:

Five feed types were selected to represent a wide range of expected starch digestibility

- Corn Silage
- High Moisture Corn
- Sorghum Silage
- Cracked Corn
- Milo/ Cracked Corn

Subsamples of each feed type were processed at three grind sizes for *In Situ* Analysis

- Coarse (Unground)
- Medium (6mm)
- Fine (4mm)

Subsamples of each feed type were processed at three grind sizes for *In Vitro* Analysis

- Coarse (6mm)
- Medium (4mm)
- Fine (1mm)

Samples were assayed for Dry Matter Digestibility and Starch Digestibility using *In Situ*, and *In Vitro* Methodology. A modified *in vitro* method with an additional filtering step was also added. Treatments are abbreviated as follows:

- IS (*In Situ*)
- IV (*In Vitro*)
- IVF (*In Vitro* with Filtering)

Samples were assayed in duplicate across three weekly runs for 3, 7, and 24 h.

IV and IVF used rumen fluid pooled from three mid-lactation Holstein cows. Rumen fluid was added to pre-warmed buffered, and standardized solution.

All animals used for rumen fluid and *in situ* runs were mid-lactation Holstein cows housed at the University of Wisconsin-Madison Blaine Dairy Cattle Center. Animals were under the Animal Care and use protocol at the University of Madison-Wisconsin.

In Situ Protocol

- 3 grams of sample weighed into 50 µM pore size Dacron bags.
- In situ bags were rinsed with cold DI water for 3, 5-minute cycles.
- In situ residues pooled by sample and analyzed for starch (Hall, 2009).

In Vitro Prep All Samples

- Pre-incubation with buffered pre-media.
- 22 mL Standardized Rumen Fluid Inoculation.

In Vitro Protocol

- (Richards, 1995; Schlau, 2024).
- 30 mL 1.2 M Acetate buffer rinse before freezing to terminate fermentation.
- 1 h boiling with 400 µL of α-amylase.
- 782.4 U of amyloglucosidase/ flask incubate at 50°C for 2 hours.
- Glucose quantified using YSI.
- Starch disappearance was calculated by dividing the residue starch by the original amount of starch and multiplying by 100.

Results:

Table 1. 7 Hour dry matter digestion means for each feed type organized by grind size[1] and digestion methodology[2]

Feed Type	Coarse			Medium			Fine		
	IS	IV	IVF	IS	IV	IVF	IS	IV	IVF
Corn Silage	46.2	.	31.4	56.0	.	34.8	57.7	.	43.0
Cracked Corn	24.3	.	15.4	30.3	.	18.1	38.0	.	32.0
HMC	59.7	.	31.9	78.4	.	36.8	78.7	.	46.7
Milo/ Cracked Corn	11.2	.	6.7	15.7	.	16.1	21.5	.	47.5
Sorghum Silage	38.5	.	25.4	43.4	.	28.9	45.4	.	36.3

[1]Coarse=6mm for IV, IVF and no grind for IS; Medium= 4mm for IV, IVF and 6mm for IS;Fine=1mm for IV,IVF and 4mm for IS

[2]IS=In Situ; IV=In Vitro ,IVF=In Vitro with Filtering

Table 2. 7 Hour starch digestion means for each feed type organized by grind size[1] and digestion methodology[2]

Feed Type	Coarse			Medium			Fine		
	IS	IV	IVF	IS	IV	IVF	IS	IV	IVF
Corn Silage	67.8	56.1	76.2	72.4	59.3	75.5	73.4	87.0	93.6
Cracked Corn	26.2	8.6	44.3	35.5	6.0	42.1	46.7	11.7	49.4
HMC	80.4	26.8	55.4	91.0	34.8	56.6	88.2	45.1	65.6
Milo/ Cracked Corn	36.2	26.2	65.0	38.8	8.5	60.6	44.4	64.7	75.2
Sorghum Silage	62.3	61.4	66.1	59.0	50.3	73.8	31.2	83.1	89.2

[1]Coarse=6mm for IV, IVF and no grind for IS; Medium= 4mm for IV, IVF and 6mm for IS;Fine=1mm for IV,IVF and 4mm for IS

[2]IS=In Situ; IV=In Vitro ,IVF=In Vitro with Filtering

Figure 1. LS means of 7 hour dry matter digestion (A, B) and starch digestion(C, D) by method and grind size.

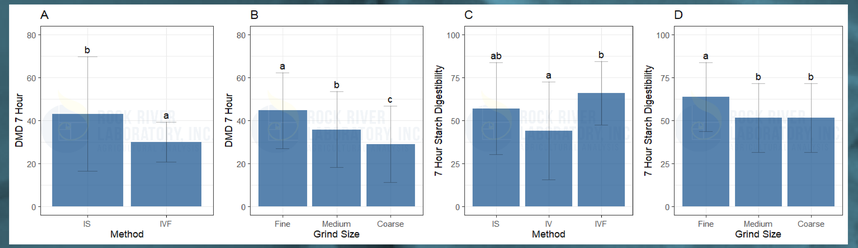

Table 3. Main model effects for 7 hour dry matter digestion and starch digestion

	DMD	Starch D
Fixed Effects	*P*-Value	*P*-Value
Run	0.44	0.79
Method	0.05	<0.01
Grind Size	<0.01	<0.01
Run: Method	0.01	<0.01

In Vitro Protocol with Filtering

- Flasks filtered on qualitative filter media, dried at 100 C, and weighed.
- Analyzed for residual starch concentration as described by Hall, 2009.
- 30mL of 0.1 M Acetate buffer (pH 5.0±0.2).
- 100 µL of α-amylase 1 hour incubation at 100°C.
- 200 U of amyloglucosidase/ flask incubate at 50°C for 2 hours.
- Glucose was quantified using YSI biochemistry analyzers.
- Residual starch was calculated using the equation:
  - 100 * [(sample volume/sample weight) *(YSI reading) * (0.9))/1000].

Statistics

- Data were analyzed using model fit function and linear mixed model approach RStudio 2025.05.0.
- Method, run, and coarseness were fixed effects and feed type was treated as a random effect.
- Interactions were evaluated and were removed from the model if not significant.
- Pairwise comparisons were evaluated using Tukey's HSD.
- 7 h data are presented due to commonality among commercial dairy samples.
- Outliers were excluded from data set and model changed between when the abstract was submitted and the poster was created.

Discussion:

Dry Matter Digestion

- Dry matter digestion cannot be calculated using the IV method.
- Run was not significant (P = 0.44).
- Method and Grind Size were significant in the model.
- LS means for IS DMD were larger than IVF (43 % vs. 30%, P = 0.05).
- DMD increased with decreasing particle size (44.7 % fine, 35.8 % medium, 29.1% coarse, P < 0.01).

Starch Digestion

- LS means for IV SD were lower than the IVF (44 % Starch vs. 65.9 % Starch, P < 0.01).
- LS means for IS method were similar to both IV and IVF method means (56.9 % Starch).
- SD means for fine grind size samples were greater than medium and coarse means (63.7 % vs. 51.5 % and 51.6 %, P < 0.01).
- IV protocol may result in lower pH than ideal for starch procedure (Hall, 2009), flasks measured 4-4.5 pH across all timepoints while acetate buffer in starch procedure is targeted at 5.0 pH.
- Particle size of residues may have confounded final starch concentration. Samples for IV and IVF with larger particle sizes may not be fully exposed for enzyme digestion during final starch determination leading to reduced final starch concentration and yielding higher IVSD results.

Conclusions:

- Methodology and grind size affect 7hr DMD and SD and should be considered when evaluating feeds across commercial applications.
- Further research should determine if these trends extend to other digestion timepoints.

References

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