Agronomic Aspects

of Silage Experimental Design and Analysis

Confounding Effects of In-Field Variability on Corn Silage Feeding Trials

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Letter to the Editor: Silage manuscripts in the *Journal of Dairy Science*

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...his is a discussion we
...d research institutes
...nists, many of them
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...g the forage sample
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...pplied. Clearly, it is
...ative forage samples
...n be achieved in sev-
...eld according to per-
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...lly tested. For more
...lations from *Animal*
... acceptable design of
silage experiments (Udén et al., 2012).

Two related factors that often come up are whether silage trials should be repeated across years or whether more than one silo per treatment is needed in silage feeding trials. There is no compelling evidence that year affects the ensiling or feeding process. Therefore, just as for feeding trials, there is no sound rationale to require silage studies to be replicated across years. Likewise, requiring replicate silos per treatment for dairy cow feeding studies is at odds with typical practice in feeding studies, where an ingredient that is being tested is from a single bay or bin from a single batch from the supplier. Although it would be ideal to ensure that ingredients tested in a feeding trial or forages examined in a silage trial truly represent all existing variability, it would be logistically challenging and cost prohibitive to do so. For example, having duplicate silos per treatment would require a doubling of the number of animals being fed a treatment, whether on trial or not, so that silo feed-out rates could be kept sufficiently high to prevent aerobic deterioration issues in one or more silages from unintentionally influencing the results of a study.

REFERENCES

Udén, P., P. H. Robinson, G. G. Mateos, and R. Blank. 2012. Use of replicates in statistical analyses in papers submitted for publication in *Animal Feed Science and Technology*. Anim. Feed Sci. Technol. 171:1–5. https://doi.org/10.1016/j.anifeedsci.2011.10.008.

5. Studies to compare treatm...

The objectives of such exper... ological treatments of feeds. Problems during review tend to occur when treatment and measurement variability is confused by authors.

The reason is that individual feeds or diets contain no variability in terms of source; only the evaluation method itself will cause variability. This variability will be higher if animals are used and lower if chemical analyses are employed.

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Feeding trials testing quality attributes of corn silage are nearly always confounded by natural, largely unavoidable variation of the quality attributes not being evaluated.

Example: Testing a practice or hybrid trait hypothesized to improve starch digestion will likely be confounded by variation in starch content, fiber digestibility, and others.

Management

Harvest Maturity
–
Chop Height
–
Processing
–
Ensiling
–
Planting Practices

CORN SILAGE YIELD & QUALITY

Environment

Water – Light – Heat

Soil – Drainage – Fertility

Field History – Disease/Pests

Brown Mid Rib
–
Hybrid

Genetics

Case Studies in Corn Silage Variability

Case Study	Description	No. of samples	%DM					Yield (tons/acre@35%DM)					% Starch					Fiber Digestibility (%NDFD24/30)					Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7)				
			Ave	Std. Dev.	Min	Max	Rng	Ave	Std. Dev.	Min	Max	Rng	Ave	Std. Dev.	Min	Max	Rng	Ave	Std. Dev.	Min	Max	Rng	Ave	Std. Dev.	Min	Max	Rng
1	Hybrid A.1, U.S.A., 2020-2024	354	36.2	4.8	25.4	54.5	29.1	26.3	6.1	7.2	43.3	36.1	36.3	5.6	17.7	52.5	34.8	51.2	4.6	40.5	65.7	25.2					
	Hybrid A.1, Northeast U.S.A, 2020-2024	207	36.0	3.8	26.7	50.1	23.4	25.8	4.9	7.9	38.2	30.3	37.0	4.9	19.0	46.9	27.9	51.5	4.8	40.5	65.3	24.8					
	Hybrid A.1, 60 mile (96 km) radius, MI, 2020-2024	48	35.4	3.5	28.6	42.6	14.0	28.2	3.6	21.0	37.1	16.1	36.4	4.6	23.5	43.5	20.0	51.9	3.9	41.4	60.3	18.9					
	Hybrid A.1, 30 mile (48 km) radius, mid-MI, 2020-2024	26	35.6	3.6	28.7	42.6	13.9	27.7	3.4	21.0	33.4	12.4	36.6	5.1	23.5	43.5	20.0	52.8	4.1	42.8	60.3	17.5					
	Hybrid A.1, 2 mile (3.2 km) radius, Gratiot, MI, 2020-2024	11	34.2	2.6	31.4	40.3	8.9	28.5	3.1	22.0	32.6	10.6	35.4	5.4	23.5	43.5	20.0	51.3	2.8	47.1	56.5	9.4					
	Hybrid A.2, ~30A (12ha) field, Gratiot, MI, 2021-2023	48	36.2	3.9	27.9	43.5	15.6	27.9	1.8	24.6	32.6	8.0	36.3	2.9	29.6	42.2	12.6	59.6	1.9	54.8	63.5	8.7	62.2	2.9	56.8	68.0	11.2
	Hybrid A vs hybrids ±2%DM & ≥20 comps, U.S.A. 2020-202	12 (313)	-0.9	0.6	-1.7	0.4	2.1	0.2	1.6	-4.9	1.6	6.5	-1.0	1.1	-2.3	1.2	3.5	-1.2	1.4	-3.0	1.1	4.1					
2	Hybrid B.1, <130 mile radius, MI, 2009	14						28.8	5.0	20	36.5	16.5	30.2	3.9	22.9	35.7	12.8	46.8	1.7	43.9	49.5	5.6					
	Hybrid B.2 (silo face grid), 60A field, 2d P/H, MI, 2010	7	31.3	0.4	30.7	32.0	1.3						30.8	1.1	28.9	32.9	4.0	47.0	3.4	41.6	51.0	9.4					
	>10 hybrids (silo face grid), >2,000 acres, 3wk P/H, MI, 201	18	33.2	2.3	28.9	37.8	8.9						31.3	3.6	25.7	37.6	11.9	46.4	3.0	40.4	51.1	10.7					
3	Hybrid C, 30 mile (48 km) radius, MI, 2015-2016	9	35.3	3.6	30.8	41.2	10.4	26.9	3.4	22.9	34.2	11.3	38.7	5.8	32.3	51.4	19.1	53.1	5.2	41.7	58.9	17.2	57.7	5.1	49.5	64.2	14.7
4	Hybrid D, 155A (63ha) field, OH, 2016	8	32.4	2.2	29.3	36.8	7.5						31.9	1.8	29.5	34.7	5.2	63.4	1.7	61.2	65.7	4.5	61.2	2.0	58.3	65.6	7.3
	Hybrid E, 120A (49ha) field, OH, 2016	8	34.9	1.3	32.9	36.9	4.0						32.1	3.8	25.4	38.7	13.3	61.6	2.7	57.1	64.6	7.5	57.4	2.9	50.6	60.7	10.1
5	Hybrid F, JD HarvestLab (~124 ft^2, 11 m^2 grids); 32A (13ha) field, MI, 2024	12,924	37.6	2.3	25.2	47.7	22.5	23.8	4.6	2.6	44.0	41.4	37.1	2.5	26.0	48.0	22.0										

Case Studies in Corn Silage Variability

Case Study	Description	No. of samples	% Starch Ave	% Starch Std. Dev.	% Starch Min	% Starch Max	% Starch Rng	Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7) Ave	Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7) Std. Dev.	Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7) Min	Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7) Max	Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7) Rng
1	Hybrid A.1, U.S.A., 2020-2024	354	36.3	5.6	17.7	52.5	34.8					
	Hybrid A.1, Northeast U.S.A, 2020-2024	207	37.0	4.9	19.0	46.9	27.9					
	Hybrid A.1, 60 mile (96 km) radius, MI, 2020-2024	48	36.4	4.6	23.5	43.5	20.0					
	Hybrid A.1, 30 mile (48 km) radius, mid-MI, 2020-2024	26	36.6	5.1	23.5	43.5	20.0					
	Hybrid A.1, 2 mile (3.2 km) radius, Gratiot, MI, 2020-2024	11	35.4	5.4	23.5	43.5	20.0					
	Hybrid A.2, ~30A (12ha) field, Gratiot, MI, 2021-2023	48	36.3	2.9	29.6	42.2	12.6	62.2	2.9	56.8	68.0	11.2

3 Hybrid C, 30 mile (48 km) radius, MI, 2015-2016

% Starch					Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7)				
Ave	Std. Dev.	Min	Max	Rng	Ave	Std. Dev.	Min	Max	Rng
38.7	5.8	32.3	51.4	19.1	57.7	5.1	49.5	64.2	14.7

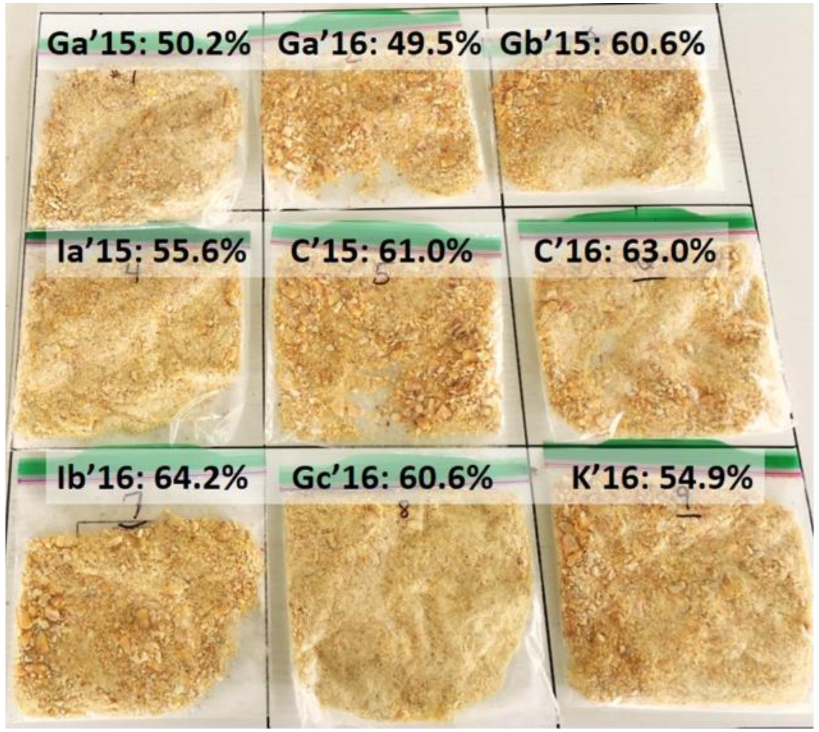
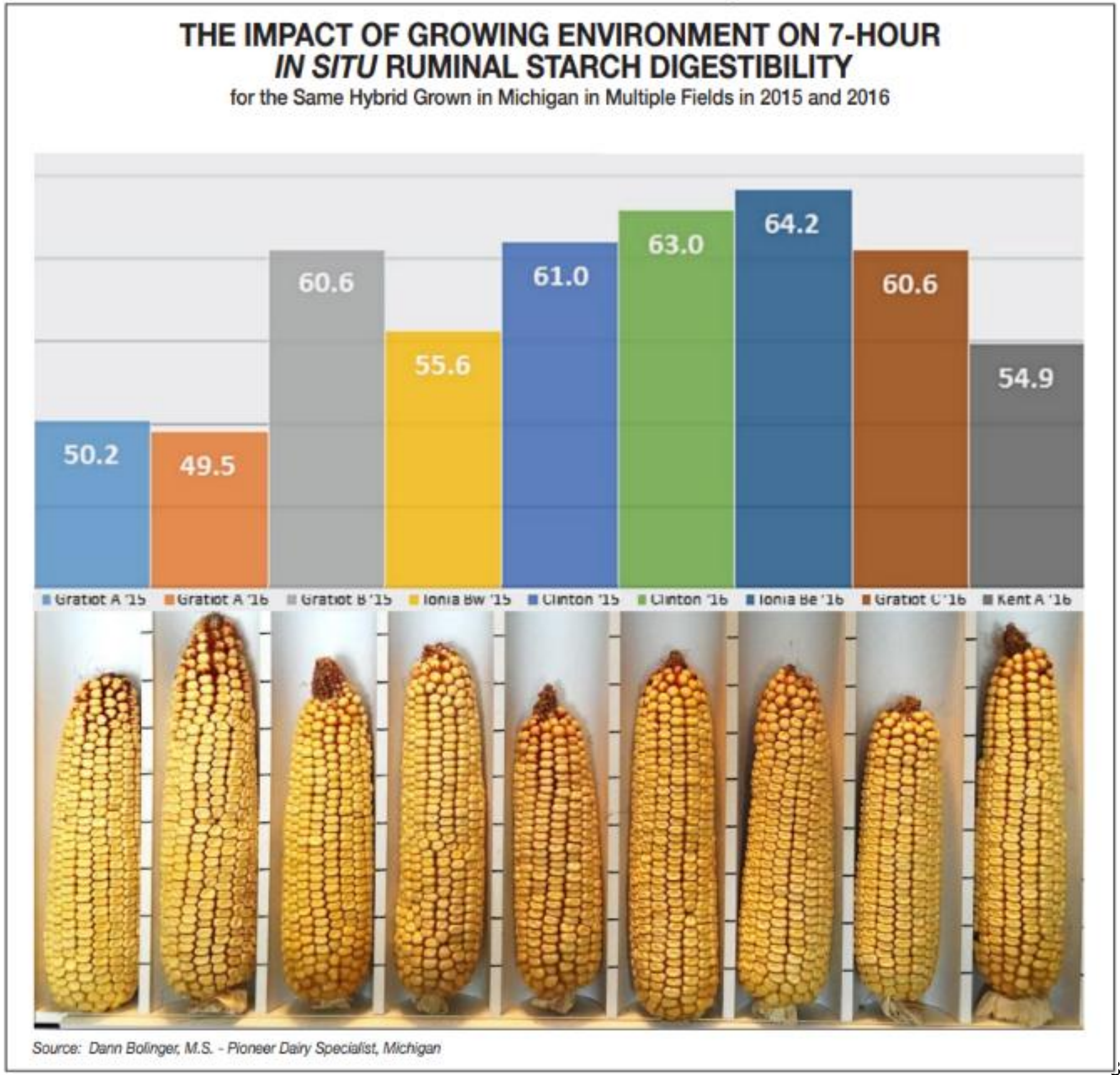


Figure 4: Ground samples of equal kernel volume and grind time with respective %ISSD-7.



Case Study	Description	No. of samples	% Starch Ave	Std. Dev.	Min	Max	Rng	Starch Dig., Pre-Ensiling (%IVSD7/%ISSD7) Ave	Std. Dev.	Min	Max	Rng
4	Hybrid D, 155A (63ha) field, OH, 2016	8	31.9	1.8	29.5	34.7	5.2	61.2	2.0	58.3	65.6	7.3
	Hybrid E, 120A (49ha) field, OH, 2016	8	32.1	3.8	25.4	38.7	13.3	57.4	2.9	50.6	60.7	10.1

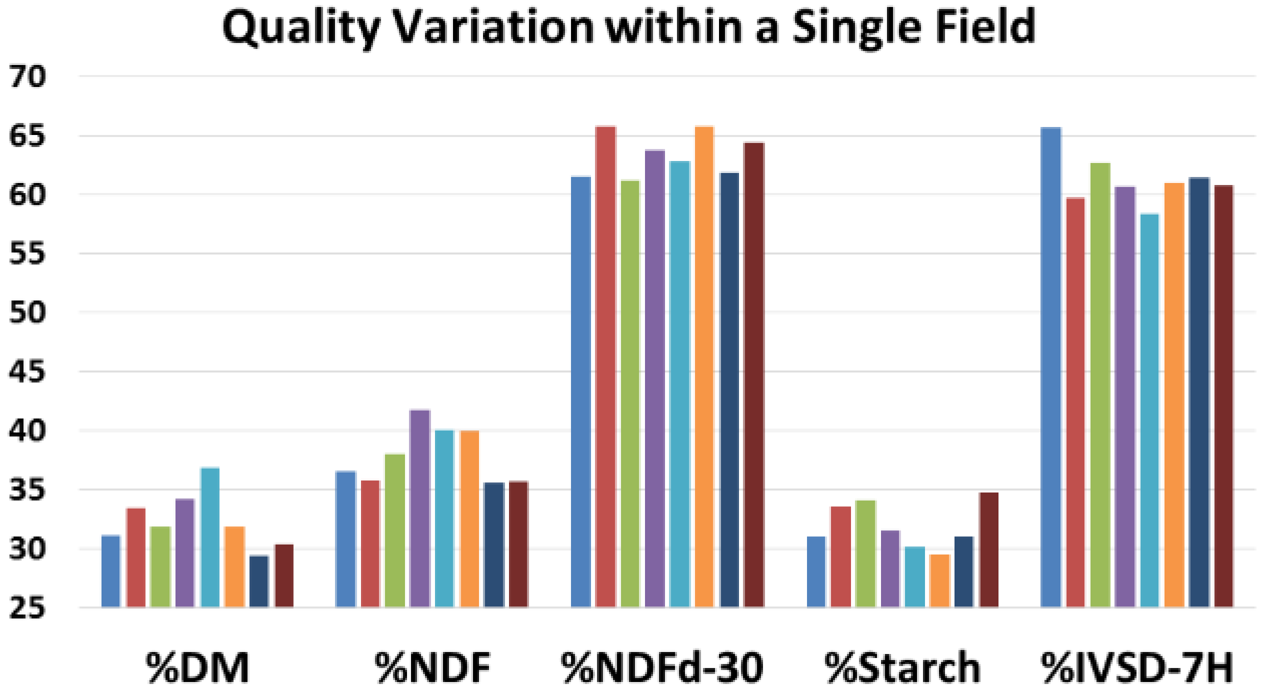
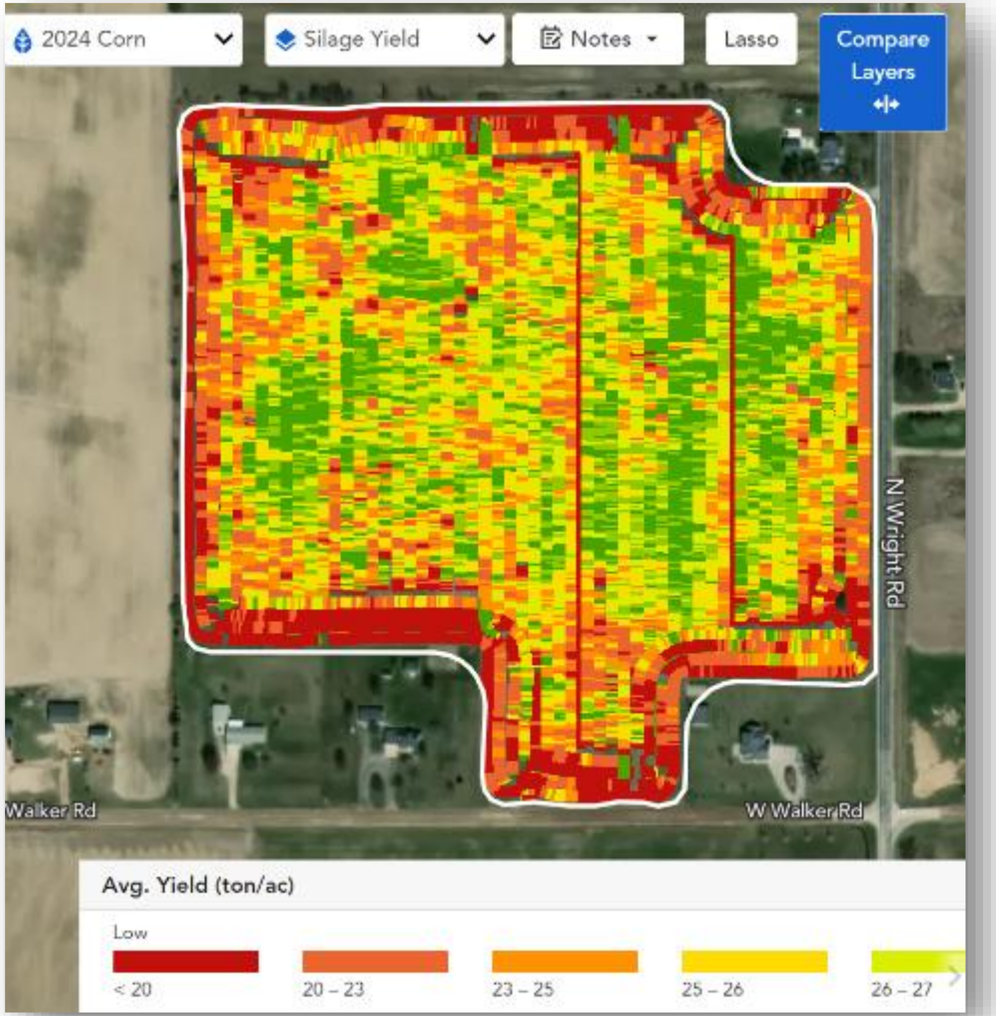


Figure 1: Quality analyses of representative samples from 8 random loads of 155 acre field, 93% by area one soil type, one hybrid, one planting date, one harvest date. (Pioneer, Ohio, 2016)

Case Study #5



32A (13 ha); 12,924 samples of ~124 ft^2 (11 m^2)

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Case Study of In-Field Variability*

32 acre field, 2 very similar highly productive, minimal slope soil-types
(13 ha)

	Average	Std. Dev.	Min.	Max.
Yield (DM T/A)	8.9	1.7	1.0	15.0
%DM	37.6	2.3	25.2	47.7
% Starch	37.1	2.5	26.0	48.0

*John Deere HarvestLab NIRS. Obviously errant values excluded. n=12,924

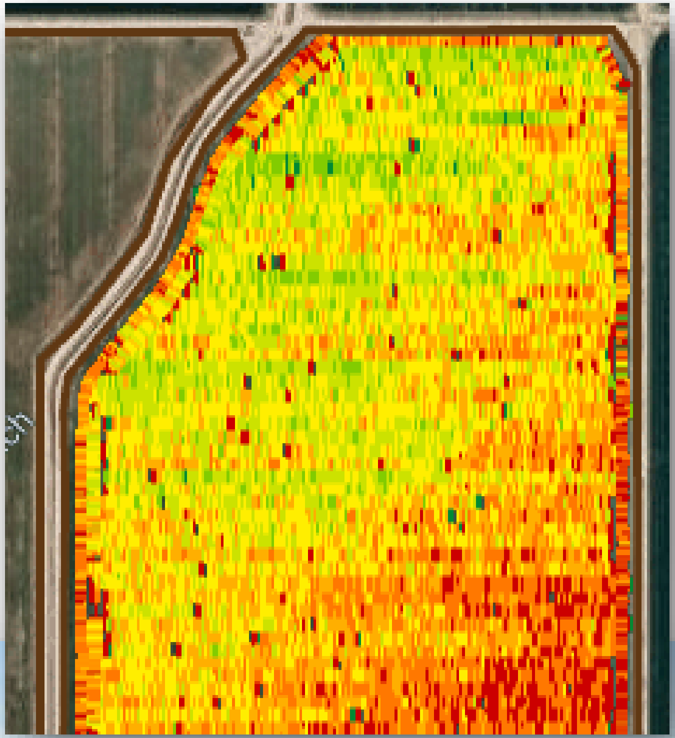
Case Study of In-Field Variability*

32 acre field, 2 very similar highly productive, minimal slope soil-types (13 ha)

Variable 1	Variable 2	Correlation, R^2
Yield (DM T/A)	%DM	0.21
Yield (DM T/A)	%Starch	0.33
%Starch	%DM	0.53

*John Deere HarvestLab NIRS. Obviously errant values excluded. n=12,924

Case Study #6: In-Field Variability - Starch



**Starch data imagery courtesy of John Deere*

Field variability patterns vary by growing season

Kharel, T.P., A. Maresma, K.J. Czymmek, E. K. Oware, and Q. M. Ketterings. 2019. Combining Spatial and Temporal Corn Silage Yield Variability for Management Zone Development. Agron. J. 111:1-9.

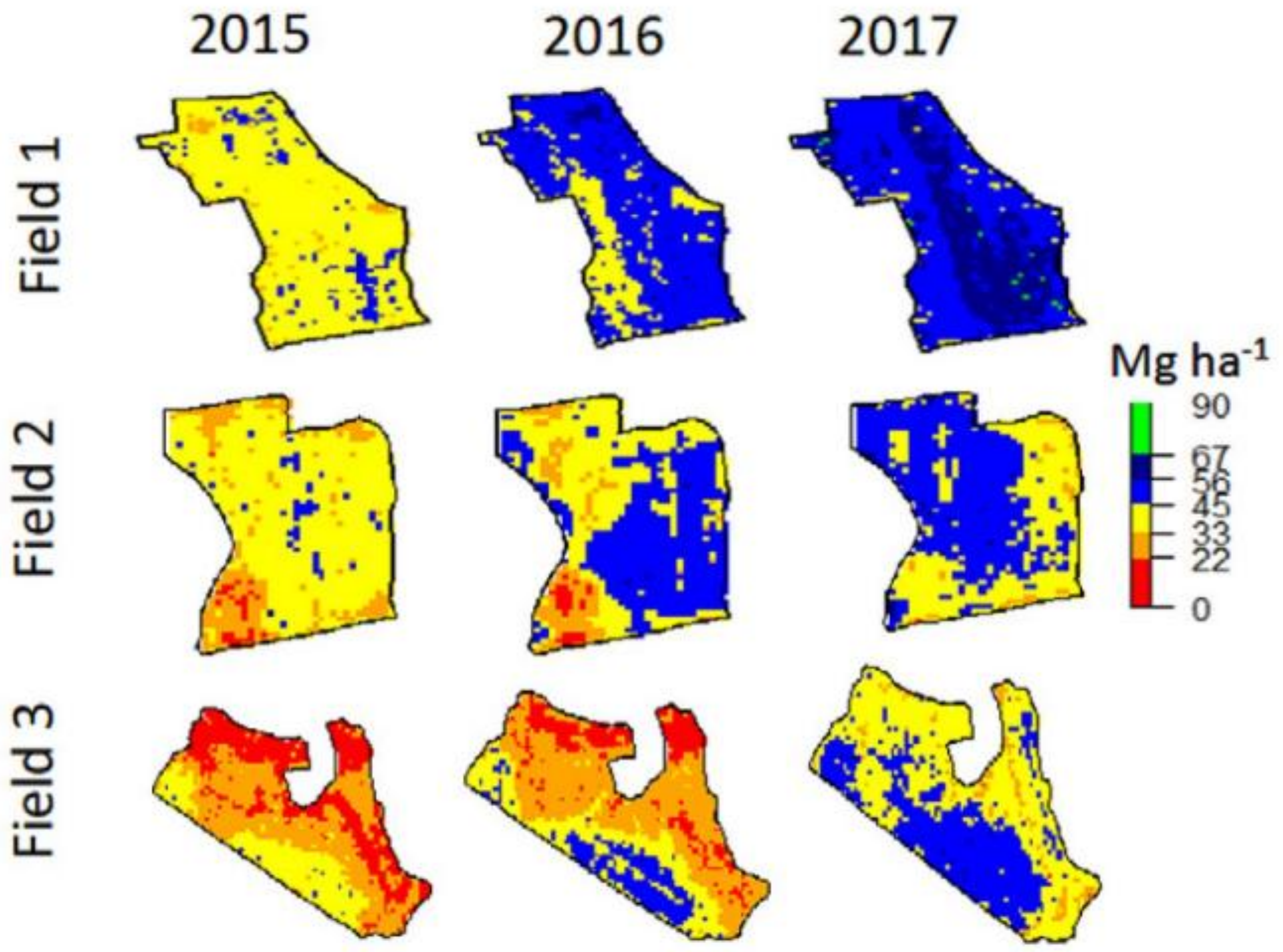


Fig. 2. Example of three fields showing the temporal and spatial yield variability of corn silage.

Amylase Corn Silage, Study #1

	Isoline	Traited	P-value
%NDFD30	31.3	30.9	0.94
% Starch	33.1	36.1	0.006
Starch Dig.,%ISSD7	**82.4**	**80.7**	**0.43**
DMI, kg/d	25.4	25.2	0.55
Milk yield, kg/d	38.8	40.8	<0.001
ECM, kg/d	38.1	39.5	0.12
ECM feed efficiency	1.51	1.57	0.05
CH4/MY	11.1	10.3	0.007

Welchez*, S. F. C., H. Stefenoni, A. Melgar, C. F. A. Lage, S. E. Räisänen, D. Wasson, M. E. Fetter, and A. N. Hristov. 2020. Effect of high-amylase corn silage on lactational performance and enteric methane emission in dairy cows. J. Dairy Sci. 103 (Suppl. 1): 68.

Amylase Corn Silage, Study #2

	Isoline	Traited	Starch=	P-value
%NDFD30	59.2	62.7		0.09[1]
% Starch	37.1	30.0		NR
Starch Dig.,%ISSD7	**81.2**	**82.1**		**0.64[1]**
DMI, kg/d	25.2	28.0	26.2	0.20
Milk yield, kg/d	36.3	35.5	36.1	0.58
ECM, kg/d	39.4	39.1	38.5	0.31
ECM feed efficiency	1.59[a]	1.45[b]	1.46[ab]	0.17
CH4/MY	12.6[b]	13.7[a]	13.1[a]	<0.05

[1] *P-values for effective rumen degradability.*

Cueva, S. F., H. Stefenoni, A. Melgar, S. E. Räisänen, C. F. A. Lage, D. E. Wasson, M. E. Fetter, A. M. Pelaez, G. W. Roth, and A. N. Hristov. 2021. Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage. J. Dairy Sci. 104:9827–9841. https://doi.org/10.3168/ jds.2021-20251.

Quality attribute	Influencing factors	Reduction practices
Dry matter content (%DM)	Plant health, harvest timing, moisture stress	Field selection, assure ample fertility via current grid soil testing and variable rate fertilizer application, verify uniform seed bed preparation and planter unit functionality, alternating treatment strips in field, plant same date, harvest same date & time, strip orientation to sub-surface drainage, irrigation, foliar fungicides, in-season nitrogen management, use a highly dominant silage inoculant, scout field throughout growing season to document visual signs of variability (consider using Normalized Difference Vegetation Index imagery, NDVI)
Starch content	Moisture stress, K uptake & timing, N uptake & timing, plant health, harvest kernel maturity, harvest cut height	
Fiber digestibility (%NDFD)	Plant health, harvest timing, K uptake & timing, N uptake & timing, moisture stress	
Starch digestibility	N uptake & timing, harvest kernel maturity, kernel processing, relative dry matter of fodder to grain	
Protein content	N uptake & timing, moisture stress, plant health	

SUMMARY

- **Compelling evidence does exist for confounding corn silage variability.**
- **Variation can be proactively reduced, but cannot be eliminated.**
- **Replication of silage production portion of experiments is obvious solution.**
  - Typically cost prohibitive, plus practical limitations.

SUGGESTED ACTIONS

- **Expand experimental design to include production of silage treatments.**
  - Alternating treatments in-field (*e.g.* split-planter) likely most practical, effective option.
- **Don't assume a valid control corn silage.**
  - Identify and acknowledge confounding quality differences in analyses and conclusions.
- **Adjust experimental diets for confounding corn silage quality variation.**
  - For example, should diets be "fermentable starch neutral" like is done for other nutrients, like nitrogen, that are held equal in experimental design?

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