27th Distillers Grain Symposium

DDGS Proficiency Testing Program / Standards Development

Chris McCullough & Len Morrissey | 09Aug-2023 | Des Moines, Iowa

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Agenda

- ASTM DDGS Proficiency Test Program Details
- DDGS PTP Cycle Review
- ASTM Standards Development

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ASTM DDGS Proficiency Testing

Program Details

- 2 Cycles per year (March, August) $788/yr
- 3 x 300 g samples per cycle
- Upon completion of testing, each laboratory submits their own data online, electronically to ASTM for use in generating electronic statistical summary reports that contain:
- Coded laboratory test results
- Statistical analyses of test data
- Charts plotting test results versus laboratory code
- Test instructions and data report forms are distributed electronically to each participant on the date samples are distributed. Labs have approximately 8 weeks to submit test data with the final statistical summary reports being electronically distributed in approximately 25 business days.
- https://www.astm.org/ptpddgs2023.html

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ASTM DDGS Proficiency Testing

Test List

- Amylase-treated Neutral Detergent Fiber (NDF)
- Acid Detergent Fiber (ADF)
- Ash
- Color (CIE Coordinates)
- Crude Protein, Fat and Fiber
- Loose Bulk Density
- Particle Size
- Moisture
- Mycotoxins (Aflatoxins, DON, Fumonisins, Ochratoxin, T-2 Toxin, HT-2 Toxin, Zeralenone)
- pH
- Water Activity

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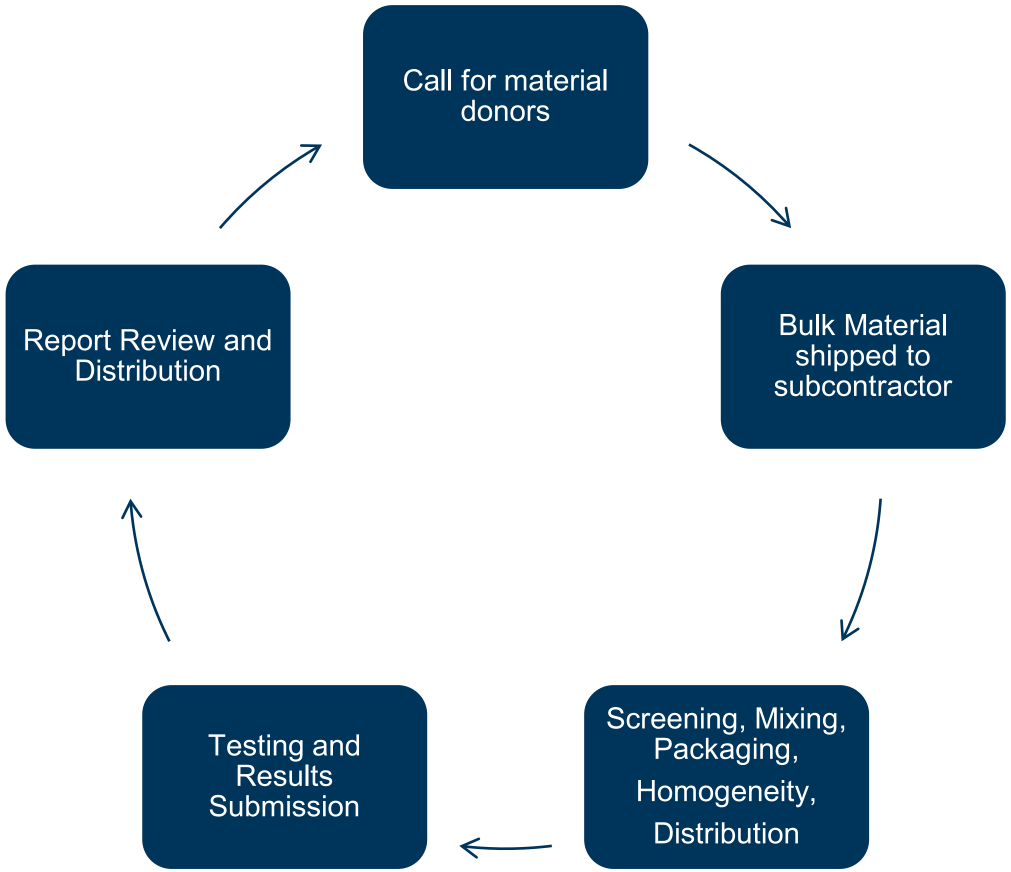
Cycle Process and Call for Material Donations



- Call for Material Donors
  - Bulk Material Shipped to Subcontractor Cost of shipment paid for by ASTM International
- Screening Tests (Protein and Moisture)
- Mixing and distribution using Boerner Divider
- Packaging in sealed double bagged
- Homogeneity Testing – to validate between sample homogeneity (Protein)
- Sample Distribution – domestic shipping included in registration fee. Options for international participants.

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DDGS PTP Cycle Review

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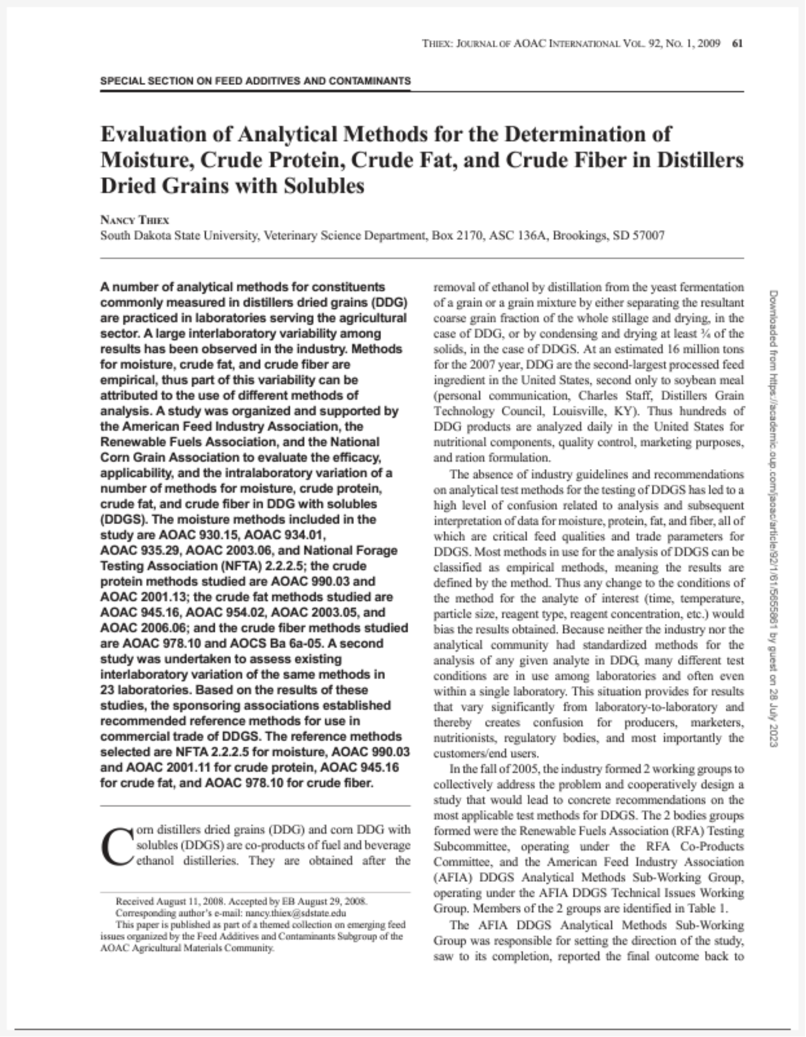
References

Thiex, N. 2012. Analytical Methodology for Quality Standards and Other Attributes of DDGS In: Distillers Grain Production, Properties, and Utilization, ed. K. Liu and K.A. Rosentrater, CRC Press, Boca Raton, FL, p. 193-217.



Thiex: Journal of AOAC International Vol. 92, No. 1, 2009 61

SPECIAL SECTION ON FEED ADDITIVES AND CONTAMINANTS

Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles

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A number of analytical methods for constituents commonly measured in distillers dried grains (DDG) are practiced in laboratories serving the agricultural sector. A large interlaboratory variability among results has been observed in the industry. Methods for moisture, crude fat, and crude fiber are empirical, thus part of this variability can be attributed to the use of different methods of analysis. A study was organized and supported by the American Feed Industry Association, the Renewable Fuels Association, and the National Corn Grain Association to evaluate the efficacy, applicability, and the intralaboratory variation of a number of methods for moisture, crude protein, crude fat, and crude fiber in DDG with solubles (DDGS). The moisture methods included in the study are AOAC 930.15, AOAC 934.01, AOAC 935.29, AOAC 2003.06, and National Forage Testing Association (NFTA) 2.2.2.5; the crude protein methods studied are AOAC 990.03 and AOAC 2001.13; the crude fat methods studied are AOAC 945.16, AOAC 954.02, AOAC 2003.05, and AOAC 2006.06; and the crude fiber methods studied are AOAC 978.10 and AOCS Ba 6a-05. A second study was undertaken to assess existing interlaboratory variation of the same methods in 23 laboratories. Based on the results of these studies, the sponsoring associations established recommended reference methods for use in commercial trade of DDGS. The reference methods selected are NFTA 2.2.2.5 for moisture, AOAC 990.03 and AOAC 2001.11 for crude protein, AOAC 945.16 for crude fat, and AOAC 978.10 for crude fiber.

Corn distillers dried grains (DDG) and corn DDG with solubles (DDGS) are co-products of fuel and beverage ethanol distilleries. They are obtained after the removal of ethanol by distillation from the yeast fermentation of a grain or a grain mixture by either separating the resultant coarse grain fraction of the whole stillage and drying, in the case of DDG, or by condensing and drying at least ¾ of the solids, in the case of DDGS. At an estimated 16 million tons for the 2007 year, DDG are the second-largest processed feed ingredient in the United States, second only to soybean meal (personal communication, Charles Staff, Distillers Grain Technology Council, Louisville, KY). Thus hundreds of DDG products are analyzed daily in the United States for nutritional components, quality control, marketing purposes, and ration formulation.

The absence of industry guidelines and recommendations on analytical test methods for the testing of DDGS has led to a high level of confusion related to analysis and subsequent interpretation of data for moisture, protein, fat, and fiber, all of which are critical feed qualities and trade parameters for DDGS. Most methods in use for the analysis of DDGS can be classified as empirical methods, meaning the results are defined by the method. Thus any change to the conditions of the method for the analyte of interest (time, temperature, particle size, reagent type, reagent concentration, etc.) would bias the results obtained. Because neither the industry nor the analytical community had standardized methods for the analysis of any given analyte in DDG, many different test conditions are in use among laboratories and often even within a single laboratory. This situation provides for results that vary significantly from laboratory-to-laboratory and thereby creates confusion for producers, marketers, nutritionists, regulatory bodies, and most importantly the customers/end users.

In the fall of 2005, the industry formed 2 working groups to collectively address the problem and cooperatively design a study that would lead to concrete recommendations on the most applicable test methods for DDGS. The 2 bodies groups formed were the Renewable Fuels Association (RFA) Testing Subcommittee, operating under the RFA Co-Products Committee, and the American Feed Industry Association (AFIA) DDGS Analytical Methods Sub-Working Group, operating under the AFIA DDGS Technical Issues Working Group. Members of the 2 groups are identified in Table 1.

The AFIA DDGS Analytical Methods Sub-Working Group was responsible for setting the direction of the study, saw to its completion, reported the final outcome back to

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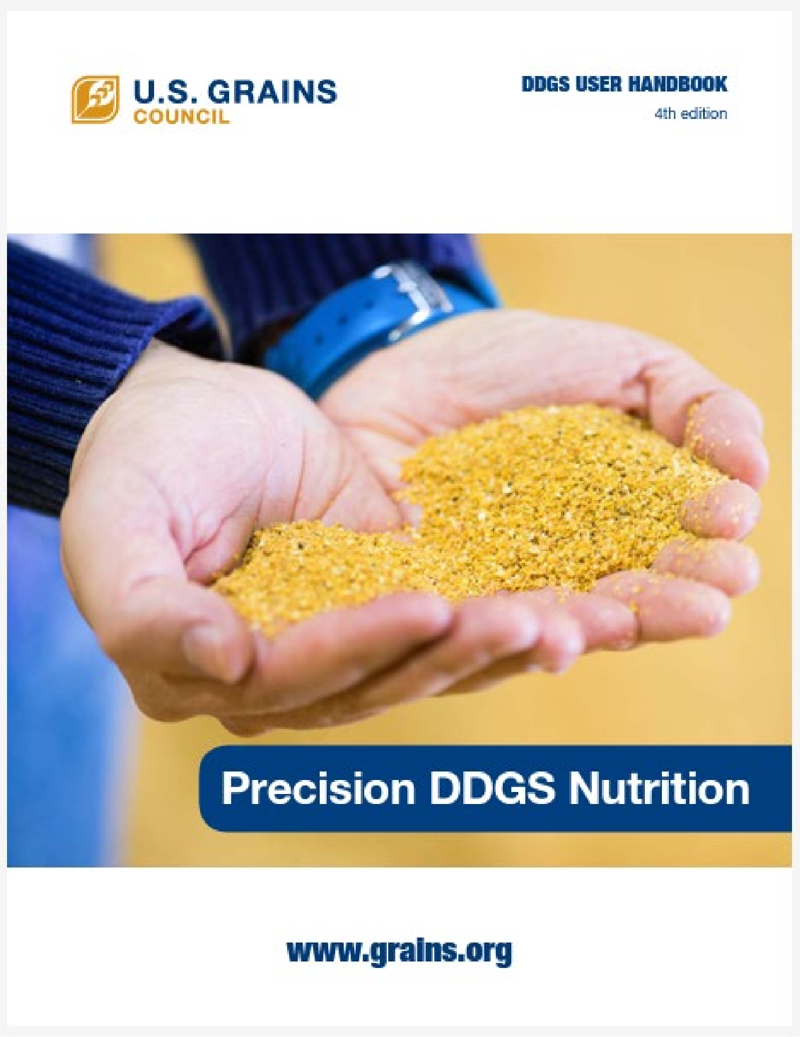

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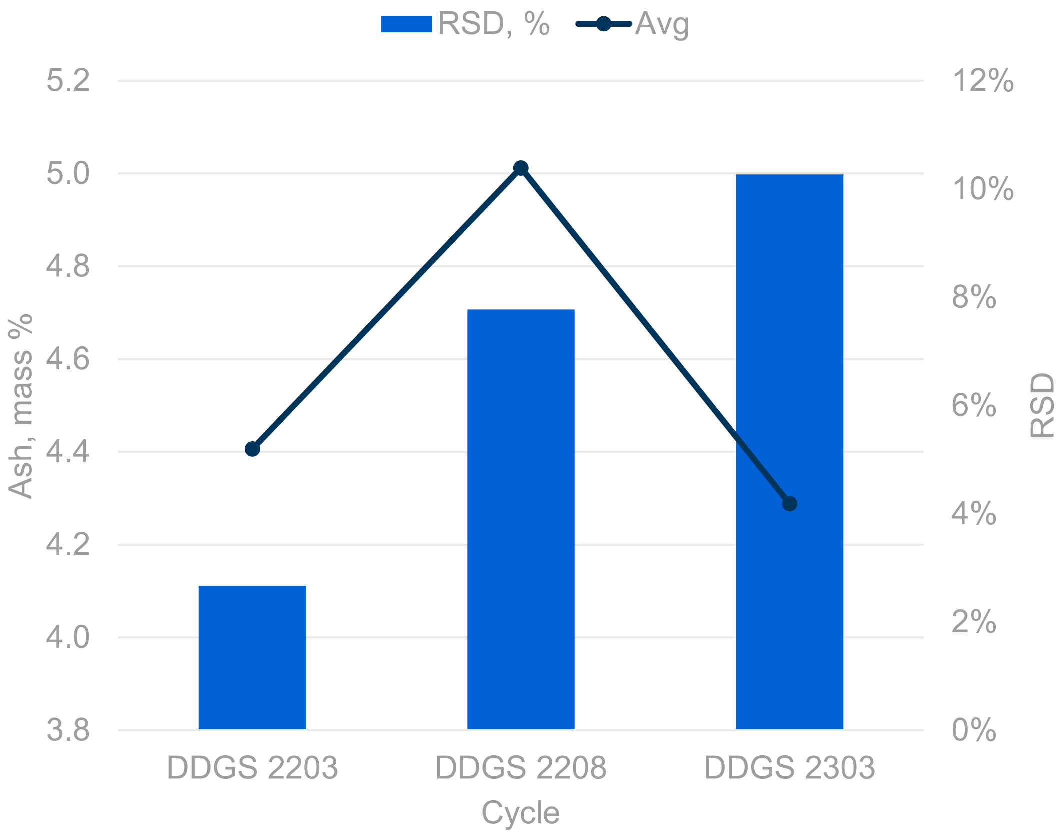
Ash

Ash (As Is), mass %												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	6	6	6	6.0	8	8	8	8.0	14	14	14	14.0
Outliers	0	0	0	0.0	0	0	0	0.0	2	2	2	2.0
Mean	4.388	4.448	4.383	4.406	5.079	4.983	4.974	5.012	4.34	4.294	4.231	4.288
Std Dev	0.101	0.124	0.127	0.117	0.404	0.4	0.365	0.390	0.475	0.414	0.432	0.440
RSD, %	2.3%	2.8%	2.9%	2.7%	8.0%	8.0%	7.3%	7.8%	10.9%	9.6%	10.2%	10.3%
A-D	0.40	0.32	0.68	0.47	0.37	0.43	0.34	0.38	0.44	0.31	0.24	0.33

Ash



– Comments:

- Methods utilized: *AOAC 942.05, (ISO 5984), Other
- Variation – likely due to different oven temperatures and times
- Action Plan
  - Request participants to submit oven temperatures and times

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Color (CIE Coordinates)

Color (CIE Coordinate L*)												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	6	7	6	6.3	7	7	7	7.0	34	34	34	34.0
Outliers	0	0	0	0.0	0	0	0	0.0	2	1	2	1.7
Mean	63.623	63.056	63.383	63.354	59.787	59.640	59.567	59.665	49.399	49.904	49.493	49.599
Std Dev	0.915	0.508	0.971	0.798	6.125	6.072	5.699	5.965	0.986	1.428	1.158	1.191
RSD, %	1.4%	0.8%	1.5%	1.3%	10.2%	10.2%	9.6%	10.0%	2.0%	2.9%	2.3%	2.4%
A-D	0.56	0.54	0.48	0.53	0.94	0.91	1.02	0.96	1.02	0.68	0.82	0.84
Color (CIE Coordinate a*)												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	3	3	3	3.0	5	5	5	5.0	27	27	27	27.0
Outliers				#DIV/0!				#DIV/0!	2	2	2	2.0
Mean				#DIV/0!				#DIV/0!	14.576	14.509	14.594	14.560
Std Dev				#DIV/0!				#DIV/0!	0.314	0.353	0.337	0.335
RSD, %	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	2.2%	2.4%	2.3%	2.3%
A-D				#DIV/0!				#DIV/0!	0.29	0.35	0.30	0.31
Color (CIE Coordinate b*)												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	3	3	3	3.0	5	5	5	5.0	27	27	27	27.0
Outliers				#DIV/0!				#DIV/0!	0	0	0	0.0
Mean				#DIV/0!				#DIV/0!	38.553	38.813	38.564	38.643
Std Dev				#DIV/0!				#DIV/0!	1.468	1.512	1.561	1.514
RSD, %	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	3.8%	3.9%	4.0%	3.9%
A-D				#DIV/0!				#DIV/0!	0.63	0.25	0.14	0.34

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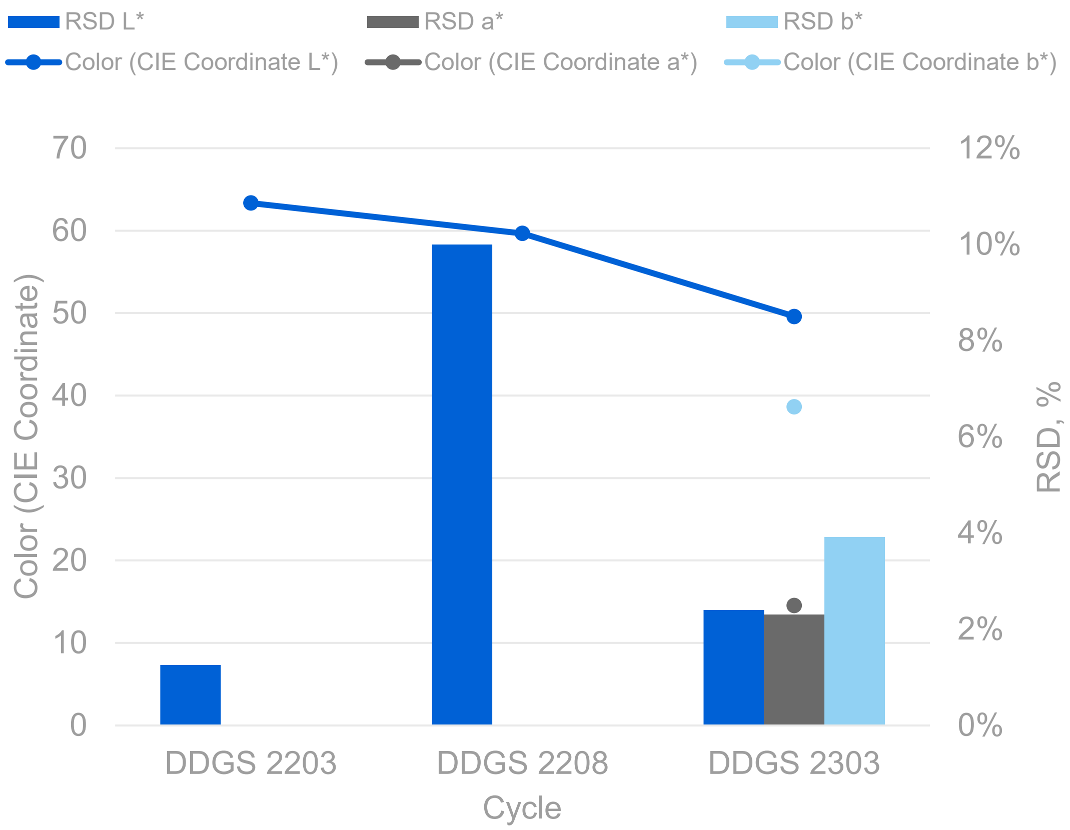
Color (CIE Coordinates)



– Comments:

- Methods utilized: Mainly Hunter Colorimeters
- Very repeatable with excellent between precision

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Crude Protein, Fat and Fiber

Crude Protein (As Is), mass %												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	7	7	7	7.0	9	9	9	9.0	35	35	35	35.0
Outliers	0	0	1	0.3	0	0	0	0.0	0	1	1	0.7
Mean	22.891	23.007	21.790	22.563	29.161	29.491	29.319	29.324	29.302	29.285	29.251	29.279
Std Dev	2.091	2.120	0.829	1.680	0.886	0.551	0.631	0.689	1.010	0.801	0.861	0.891
RSD, %	9.1%	9.2%	3.8%	7.4%	3.0%	1.9%	2.2%	2.4%	3.4%	2.7%	2.9%	3.0%
A-D	0.71	0.30	0.52	0.51	0.55	0.40	0.44	0.46	0.33	0.25	0.31	0.30
Crude Fat (As Is), mass %												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	7	7	7	7.0	8	8	8	8.0	35	35	35	35.0
Outliers	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0
Mean	6.661	6.334	6.403	6.466	7.454	7.513	7.416	7.461	6.641	6.683	6.656	6.660
Std Dev	0.926	0.957	1.172	1.018	0.630	0.528	0.401	0.520	1.013	1.089	1.031	1.044
RSD, %	13.9%	15.1%	18.3%	15.7%	8.5%	7.0%	5.4%	7.0%	15.3%	16.3%	15.5%	15.7%
A-D	0.33	0.22	0.27	0.27	0.35	0.51	0.32	0.39	1.18	0.85	0.82	0.95
Crude Fiber (As Is), mass %												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	4	4	4	4.0	6	6	6	6.0	4	4	4	4.0
Outliers				#DIV/0!	0	0	0	0.0				#DIV/0!
Mean				#DIV/0!	9.126	9.087	8.838	9.017				#DIV/0!
Std Dev				#DIV/0!	0.573	0.707	0.811	0.697				#DIV/0!
RSD, %	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	6.3%	7.8%	9.2%	7.7%	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
A-D				#DIV/0!	0.25	0.26	0.27	0.26				#DIV/0!

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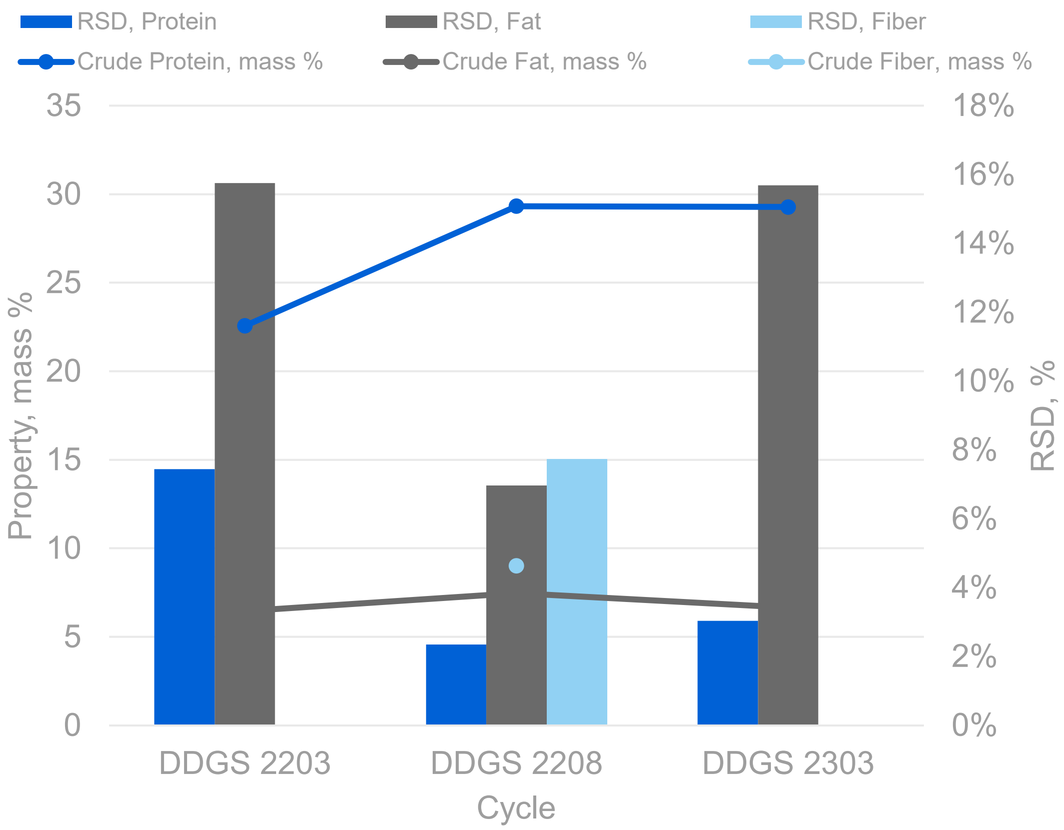
Crude Protein, Fat and Fiber



– Comments:

- Methods utilized:
  - Protein: *AOAC 990.03 & Near IR
  - Fat: *AOAC 945.16 & Near IR
  - Fiber: AOCS Ba 6a-05 (*AOAC 976.10)
- DDGS 2303 Precision was different vs Thiex paper
  - Protein RSD 3.0 % vs 0.64 %
  - Fat RSD 15.7 % vs 2.95 %
  - Fiber RSD 7.7 % (DDGS 2208) vs 4.02 %

Loose Bulk Density and Particle Size (Geometric Mean Diameter)

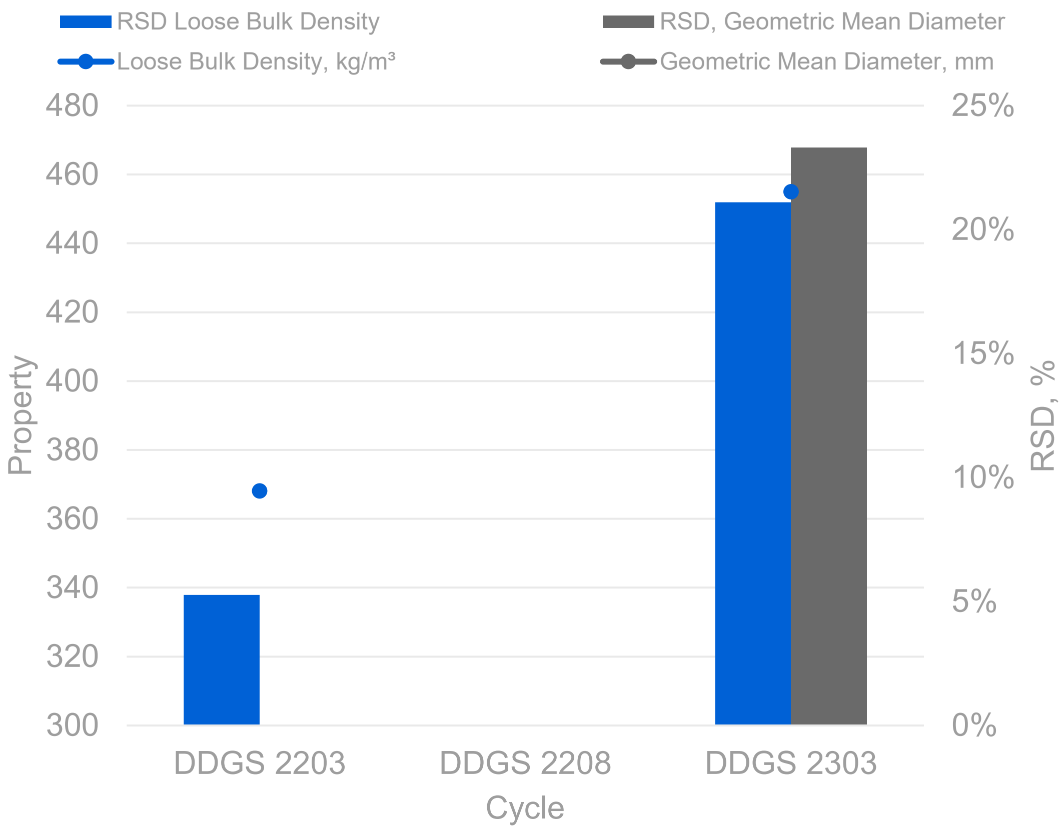
Loose Bulk Density (As Is), kg/m³

Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	8	8	8	8.0	5	5	5	5.0	24	24	24	24.0
Outliers	1	1	1	1.0				#DIV/0!	10	10	10	10.0
Mean	369.990	367.367	367.004	368.120				#DIV/0!	454.853	453.354	456.717	454.975
Std Dev	18.252	18.632	21.253	19.379				#DIV/0!	95.594	95.179	97.317	96.030
RSD, %	4.9%	5.1%	5.8%	5.3%	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	21.0%	21.0%	21.3%	21.1%
A-D	0.45	0.32	0.41	0.39				#DIV/0!				#DIV/0!

Particle Size (Geometric Mean Diameter), mm

Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	5	4	4	4.3	3	3	3	3.0	10	10	10	10.0
Outliers				#DIV/0!				#DIV/0!	4	4	4	4.0
Mean				#DIV/0!				#DIV/0!	1.095	1.255	1.083	1.144
Std Dev				#DIV/0!				#DIV/0!	0.363	0.118	0.319	0.267
RSD, %	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	33.2%	9.4%	29.5%	23.3%
A-D				#DIV/0!				#DIV/0!				#DIV/0!

Loose Bulk Density and Particle Size (Geometric Mean Diameter)



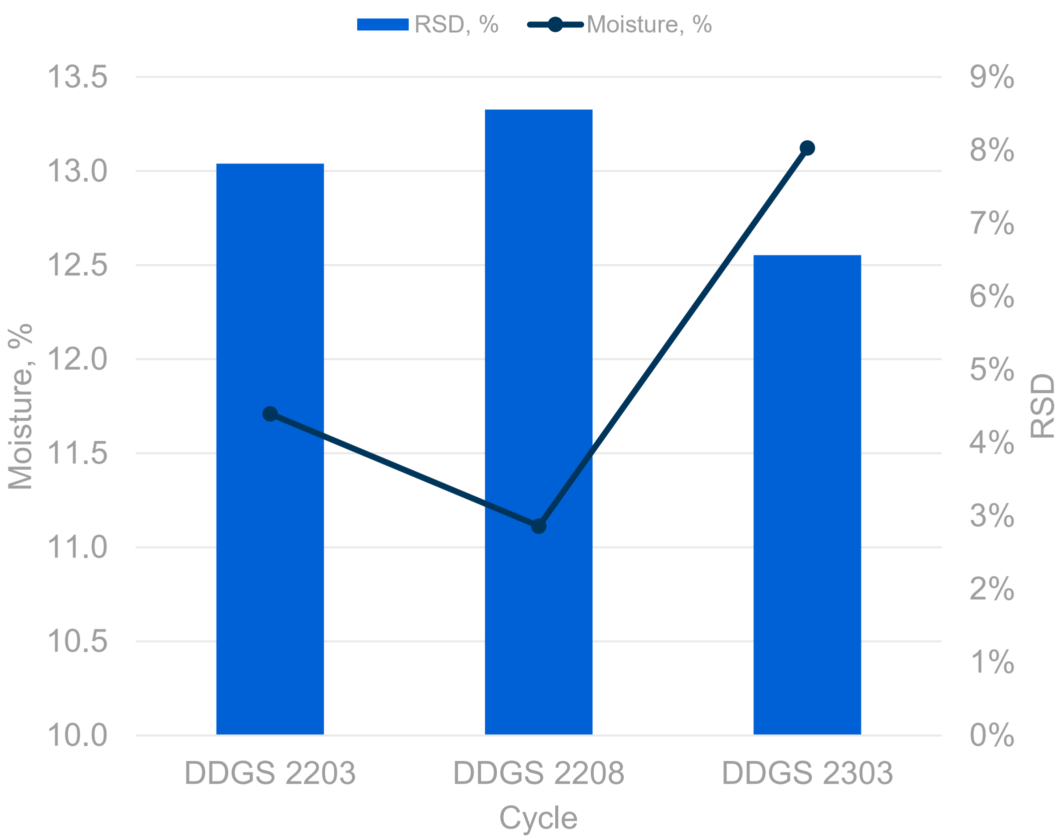
– Comments:

- Methods utilized:
- V

Moisture

Moisture, Dry Matter, %												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	13	14	13	13.3	14	14	14	14.0	44	44	44	44.0
Outliers	3	4	3	3.3	0	0	0	0.0	2	2	2	2.0
Mean	11.655	11.747	11.728	11.710	11.041	11.094	11.204	11.113	13.163	13.106	13.099	13.123
Std Dev	0.851	0.938	0.957	0.915	0.866	1.024	0.962	0.951	0.940	0.860	0.785	0.862
RSD, %	7.3%	8.0%	8.2%	7.8%	7.8%	9.2%	8.6%	8.6%	7.1%	6.6%	6.0%	6.6%
A-D	0.72	0.73	0.59	0.68	0.26	0.49	0.43	0.39	0.84	0.50	0.34	0.56

Moisture, %



– Comments:

- Methods utilized: *NFTA 2.1.4 (105℃/3hr) / (NFTA 2.2.2.5 (105℃/3hr), AOAC 935.29, Near IR
  - Is designation of NFTA 2.2.2.5 correct?
- Data Entry – update to capture oven temperature and time and split out data to capture determinations by oven drying and NIR in separate data sets.

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Aflatoxins

Aflatoxins (Total), µg/kg (ppb)												
Cycle	DDGS 2203				DDGS 2203				DDGS 2203			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	8	7	7	7.3	7	7	7	7.0	36	36	36	36.0
Outliers	0	0	0	0.0	0	0	0	0.0	1	1	0	0.7
Mean	2.521	1.900	1.666	2.029	2.741	2.151	2.527	2.473	1.596	1.565	1.679	1.613
Std Dev	2.443	1.406	1.208	1.686	2.794	1.818	3.187	2.600	0.952	0.937	1.112	1.000
RSD, %	96.9%	74.0%	72.5%	83.1%	101.9%	84.5%	126.1%	105.1%	59.6%	59.9%	66.2%	62.0%
A-D	0.81	0.24	0.70	0.583333	0.55	0.20	0.60	0.45	0.34	0.77	0.64	0.583333

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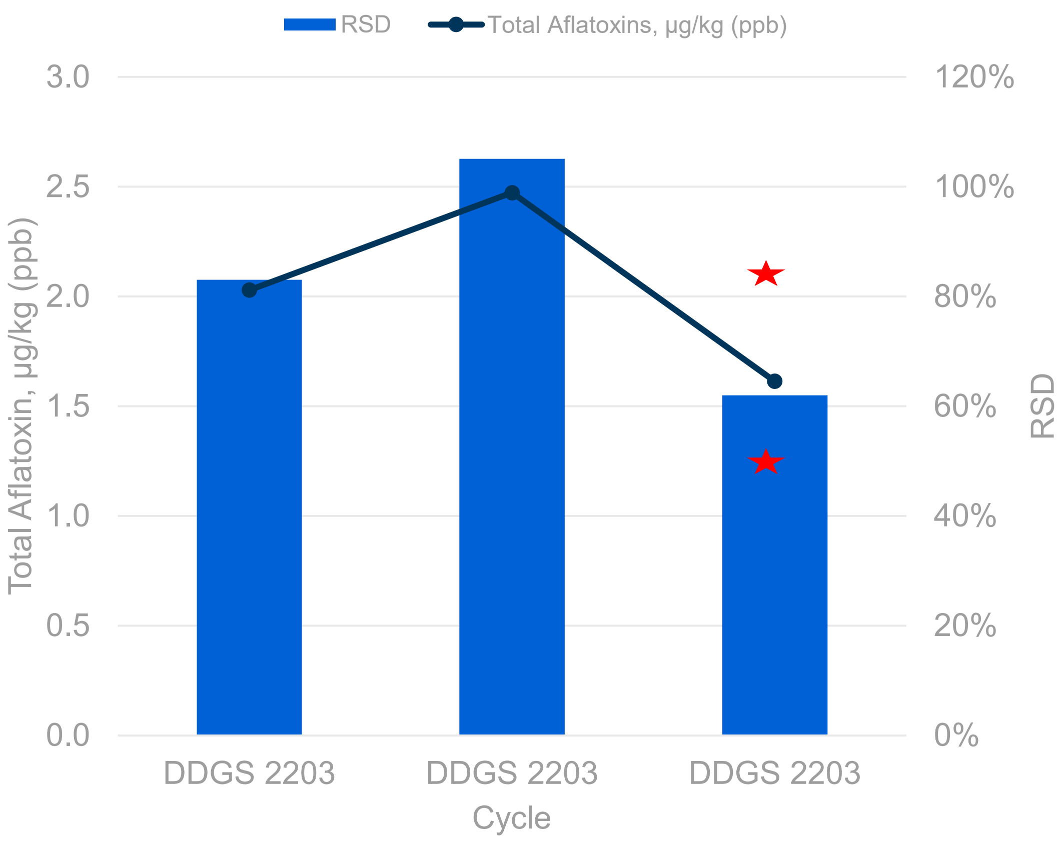
Total Aflatoxins, µg/kg (ppb)



– Comments:

- Methods utilized: (*AOAC 994.08), LC-MS/MS, Neogen Reveal Q+, Neogen Reveal Q+ Max
  - GIPSA approved test kits (USGC DDGS Handbook)
    - Veratox Aflatoxin (Neogen)
    - Ridascreen FAST SC (R-Bippharm)
    - Aflatest (Vicam)
    - FluroQuant / Afla IAC (Romer)
- Ways to improve single lab and between lab precision (high r & R)? – between sample averages very consistent
- Only two labs reported values based on LC-MS/MS and they bracket the average
- We will update data entry to capture brand/model of reader and brand/model of test kit (list)

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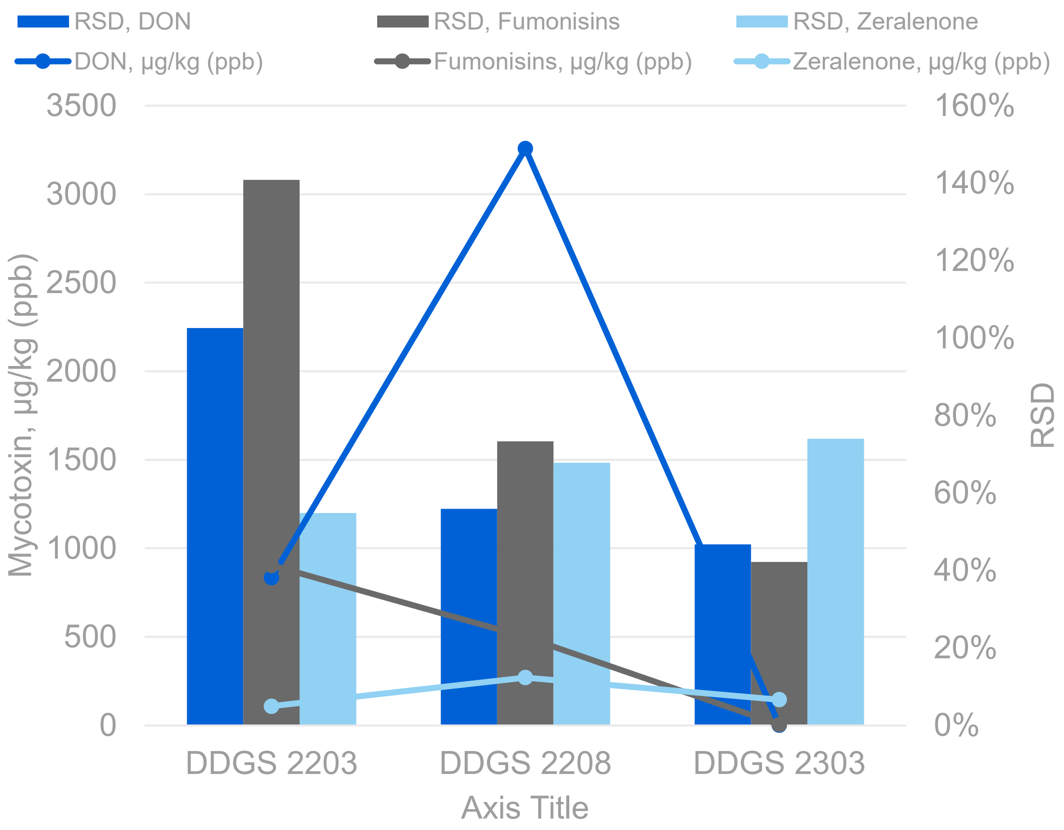
Additional Mycotoxins

DON, µg/kg (ppb)												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	8	7	7	7.3	8	8	8	8.0	38	38	38	38.0
Outliers	0	0	0	0.0	0	0	0	0.0	8	8	8	8.0
Mean	749.095	856.794	896.479	834.123	3313.599	3210.256	3247.040	3256.965	1.371	1.406	1.399	1.392
Std Dev	839.487	842.175	886.104	855.922	1800.239	1908.928	1757.538	1822.235	0.624	0.654	0.675	0.651
RSD, %	112.1%	98.3%	98.8%	102.6%	54.3%	59.5%	54.1%	55.9%	45.5%	46.5%	48.2%	46.8%
A-D	0.89	0.68	0.77	0.78	0.31	0.30	0.29	0.30				#DIV/0!

Fumonisins, µg/kg (ppb)												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	7	7	7	7.0	6	6	6	6.0	33	33	33	33.0
Outliers	0	0	0	0.0	0	0	0	0.0	7	7	7	7.0
Mean	896.169	905.523	908.503	903.398	528.413	497.735	472.940	499.696	4.283	4.431	4.299	4.338
Std Dev	1264.456	1283.709	1268.158	1272.108	381.884	337.140	380.766	366.597	2.035	1.868	1.587	1.830
RSD, %	141.1%	141.8%	139.6%	140.8%	72.3%	67.7%	80.5%	73.4%	47.5%	42.2%	300.0%	42.2%
A-D	0.97	0.96	0.94	0.96	0.16	0.30	0.33	0.26	9.00	8.15	8.05	8.40

Zeralenone, µg/kg (ppb)												
Cycle	DDGS 2203				DDGS 2208				DDGS 2303			
Sample	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
n	7	7	7	7.0	10	10	10	10.0	36	36	36	36.0
Outliers	0	0	0	0.0	0	0	0	0.0	0	2	2	1.3
Mean	113.727	109.349	103.600	108.892	278.411	279.491	253.309	270.404	172.095	130.648	133.634	145.459
Std Dev	53.948	55.844	69.396	59.729	205.665	168.507	175.652	183.275	143.340	92.238	87.341	107.640
RSD, %	47.4%	51.1%	67.0%	54.9%	73.9%	60.3%	69.3%	67.8%	83.3%	70.6%	65.4%	74.0%
A-D	0.88	0.43	0.39	0.57	0.39	0.28	0.29	0.32	2.27	2.07	0.99	1.78

Additional Mycotoxins



– Comments:

- Methods utilized: (*AOAC 2001.04), LC-MS/MS, Rapid Test Kits
  - GIPSA approved test kits (USGC DDGS Handbook)
    - AgraQuant Total Fumonisin 0.25/5.0 (Romer)
- DON repeatability pretty consistent
- Issue with reporting (ppm vs ppb)

Removals / Additions Discussion

Tests not evaluated due to insufficient data

- ADF
- NDF
- Particle Size, Geometric Standard Deviation
- Sulfur
- Aflatoxins (B2, G1, G2)
- Other Mycotoxins (Fumonisin B1, B2, B3, Ochratoxin A, T-2 Toxin, HT-2 Toxin)
- pH

Other Tests

- Acid detergent lignin (ADL) - AOAC 973.18
- Starch - No official method
- Amino acids - AOAC 995.12 or ISO 13903
- Tryptophan - AOAC 988.15
- Chlorine - AOAC 969.10 or AOAC 943.01 or ISO 6495
- Chromium - No official method
- Fluorine - Microdiffusion technique
- Iodine – ICP-MS
- Phosphorus - AOAC 965.17 or ISO 6491 or ISO 27085
- Selenium - AOAC 996.16 or AOAC 996.17
- Trace Minerals - AOAC 968.08 or ISO 6869 or ISO 27085

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Subcommittee on the manufacturing and commercial practices of Distillers Dried Grains (DDG)

Scope - The promotion of knowledge, stimulation of research, and the development of standards and methods, and development of recommended practices and guides related to the analysis of distillers grains and co-products produced from the biorefinery and distillery industries.

– Planning meeting held virtual April 5, 2023
– Unanimous approval to propose starting a new subcommittee within Committee E48 on Bioenergy and Industrial Chemicals from Biomass
– Request approved by the E48 Executive Committee
– Organizational meeting of the subcommittee held June 30th during the E48 Committee Meetings in Denver
– Next meeting – virtual August 16th
– Open to anyone with an interest.

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- Student Membership is free and open to all students – it provides a full understanding of the standardization process

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To Join Committee E48

https://www.astm.org/get-involved/membership.html

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