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*Evaluation of Analytical
Methods for Analysis of Dried
Distillers Grains with Solubles*

*AFIA Sub-Working Group
Final Report
February 1, 2007*

Co-sponsored and Equally Funded by:





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Background

Within the Fuel Ethanol Industry there are currently no guidelines or even recommendations on which analytical test methods should be used for the measurement of DDGS, which leads to a significant level of confusion related to analysis and subsequent interpretation of data for moisture, protein, fat, and fiber, all of which are critical feed quality parameters for DDGS. Most wet chemistry methods used for the analysis of DDGS in the analytical community currently are what would be classified as *empirical methods*, meaning the results are an indirect measurement of the analyte of interest and the results are in part or in whole dependent on the conditions of the assay (i.e. reagent type or concentration and assay parameters like temperature, time, pH, etc.). Since the analytical community has not yet come to a consensus on what empirical method is best suited for the analysis of any given analyte in DDGS, many different empirical methods are used amongst laboratories and even within a single laboratory. The use of various empirical methods for a single analyte leads to results that vary significantly from lab to lab and thereby create a great deal of confusion for producers, marketers, nutritionists, regulatory bodies, and most importantly the customers/end-users. *Segal's Law*, which states, "A man with a watch knows what time it is. A man with two watches is never sure", sums up the current state of affairs best as it relates to analysis of DDGS.

This problem was identified by the Ethanol Industry and strategically addressed in the Fall of 2005; two working group bodies were formed to collectively address the problem and cooperatively design a study which would lead to concrete recommendations on the most applicable test methods for DDGS. The two bodies formed to accomplish this task were:

RFA Testing Subcommittee (Operating under the *RFA Co-Products Committee*)

Members:	Shon Van Hulzen	Broin Management
	Dr. Lance Forster	ADM
	Charlie Staff	Distillers Grain Technology Council
	Bob Dinneen	Renewable Fuel Association

AFIA DDGS Analytical Methods Sub-Working Group (Operating under the *AFIA DDGS Technical Issues Working Group*)

Members:	Shon Van Hulzen	Broin Management
	Dr. Lance Forster	ADM
	Charlie Staff	Distillers Grain Technology Council
	Dr. Thomas Robb	Abengoa Bioenergy
	Dr. Phil Smith	Tyson Foods, Inc.
	Thomas Sliffe	Perten Instruments
	Trace Yates	Tyson Foods
	Mark Host	FOSS North America
	Lars Reimann	Eurofins Scientific

Shon Van Hulzen, Quality Control Director, Broin Management, was chosen as the chair for both committees.





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Nancy Thiex, Laboratory Manager, Olson Biochemistry Laboratories, was selected as the primary consultant by the AFIA group, and was the organizer, coordinator, and statistical evaluator of the study.

The RFA group was to provide input and insights from the perspective of the Ethanol Industry as well as provide several members to serve on the AFIA group, which also included several representative from the feed industry as well as other stake holder members. The AFIA DDGS Analytical Methods Sub-Working Group would also be the body responsible for setting the direction of the study, see to its completion, and reporting the final outcome and eventual recommendations based on the data.

The Study

The study was designed to evaluate the efficacy, applicability, the *intra* laboratory variation, and the *inter* laboratory variation of the most commonly used test methods in the analytical community for the analysis of Moisture/Loss on Drying, Crude Protein, Crude Fat, and Crude Fiber. Table 1 below lists the analytical methods that were evaluated in this study.

Table 1 Test Methods for DDGS

Moisture/Loss on Drying	
AOAC 934.01	Loss on Drying (Moisture) for Feeds (Vacuum Oven 95-100 °C)
AOAC 935.29	Moisture in Malt (Gravimetric Method at 103-104 °C / 5 hr)
NFTA 2.2.2.5	Lab Dry Matter (105 °C / 3 hr)
AOAC 930.15	Loss on Drying (Moisture) for Feeds (135 °C / 2 hr)
AOAC 2001.12	Determination of Water/Dry Matter (Moisture) in Animal Feed, Grain, and Forage (Karl-Fischer)
Crude Protein	
AOAC 990.03	Protein (Crude) in Animal Feed - Combustion
AOAC 2001.11	Protein (Crude) in Animal Feed and Pet Food (Copper Catalyst)
Crude Fat	
AOAC 2003.05	Crude Fat in Feeds, Cereal Grains, and Forages (Ether Ext.)
AOAC 2003.06	Crude Fat in Feeds, Cereal Grains, and Forages (Hexane Ext.)
AOAC 954.02	Crude Fat by Acid Hydrolysis
AOAC 945.16	Oil in Cereal Adjuncts (Petroleum Ether)
Crude Fiber	
AOAC 978.10	Fiber (Crude) in Animal Feed and Pet Food (F.G. Crucible)
AOCS Ba 6a-05	Ankom Method





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Phase I, which was designed to evaluate the efficacy, applicability, and the *intra* laboratory variation of the respective test methods, involved the analysis of 30 samples, which were collected from six carefully selected locations (five samples from each location); with the intention of gathering a sample set that resembles a cross section of the market. The six locations are found in the Table 2 below.

Table 2 Phase I Sample Matrix Locations

- 2 locations from Broin Companies Corn Dry Mill Plants - (2 different processes)
- 2 locations from ADM Corn Dry Mill Plants - (2 different processes)
- 1 location from an Alternative Feedstock Dry Mill (Western Plains Energy in Oakley, KS)
- 1 location from a Beverage (potable) Plant (Jim Beam)

Each of the 30 samples (5 samples from each location X 6 sample locations) was analyzed in triplicate by all of the methods listed in Table 1 above at the Olsen Biochemistry Laboratories, under the direction of Nancy Thiex. The results achieved are summarized in Table 3 below and in Figures 1-2 below.

Phase II, which was designed to evaluate the *inter* laboratory variation, involved the analysis of 5 samples, which were a subset of the samples collected for Phase I. The five samples were one sample from each of the six locations – one of the locations was unable to submit the larger sample size required for the inter laboratory portion of the study and was thereby left out of Phase II, hence the five samples in Phase II instead of the intended six samples. The five samples were sent to 23 participating laboratories and analyzed in duplicate for each method the respective laboratory had signed up for in advance. The results achieved for the five samples at the 23 participating laboratories are summarized in Table 3 below and in Figures 3-4 below.

Conclusions

All statements in the following sections are based on the statistical analysis and related conclusions found in the final report from Nancy Thiex, which can be supplied upon request by contacting either Nancy Thiex (nancy_thiex@sdstate.edu) or Shon Van Hulzen (shon.vanhulzen@broin.com). A summary of the committee's recommendations can be found in Table 4 below.

Moisture/Loss on Drying

Although it is commonly known and widely accepted that Karl Fischer Titration provides the most accurate measurement of water in feed, the labor (both time and training), reagent, and instrument costs make Karl Fischer analysis an economic burden that most laboratories would not be willing to bear. The committee recognizes these concerns and has used Karl Fischer as the means of determining the gravimetric (loss on drying) method that has the least amount of bias when compared to Karl Fischer. Using this criteria, **NFTA 2.2.2.5, Lab Dry Matter (105 °C / 3 hr), was selected as the recommended method for the analysis of moisture in DDGS**; this method also had acceptable CV's in both the intra and inter laboratory portions of the study.





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The committee also wishes to emphatically note that all gravimetric methods be considered, and used accordingly, as “loss on drying” methods and only serve as an estimation of the “true” moisture level. One of the gravimetric methods, AOAC 930.15, *Loss on Drying (Moisture) for Feeds (135 °C / 2 hr)*, was shown to dramatically overestimate the moisture content in DDGS and therefore, it is highly discouraged to use this method to analyze samples of DDGS; use of this method is widespread as demonstrated by the fact that 17 of the 23 labs reported values using AOAC930.15. Use of this method is highly discouraged and efforts to remove the method from use on DDGS should be pursued.

Protein

The protein methods investigated in this study were determined to be statistically equivalent and both had acceptable coefficients of variation for both the intra and inter laboratory portions of the study. **AOAC 990.03, Protein (Crude) in Animal Feed – Combustion, and AOAC 2001.11, Protein (Crude) in Animal Feed and Pet Food (Copper Catalyst), can therefore be used interchangeably to provide accurate and precise protein results on DDGS.**

Fat

The three non-hydrolysis fat methods (AOAC 2003.05, AOAC 945.16, and AOAC 2003.06) were determined to be statistically equivalent methods for the analysis of DDGS, however, in the inter laboratory portion of the study, **AOAC 945.16, Oil in Cereal Adjuncts (Petroleum Ether), had a significantly lower coefficient of variation than the other non-hydrolysis methods and has thereby proven to be a more robust method in the analytical community, and is therefore chosen as the recommended test method for the analysis of fat in DDGS.**

The acid hydrolysis method (AOAC 954.02) was determined to be significantly different, with a bias of ~+4% (absolute difference). It should be noted that only relative accuracy was compared and since all four methods in the investigation are empirical in nature, further work would have to be completed to determine the most accurate method. However, since the three non-hydrolysis methods were found to be statistically equivalent methods, it was decided that the most robust (most repeatable) non-hydrolysis method in the inter laboratory portion of the study would be selected as the method of choice.

Fiber

Both crude fiber methods evaluated, AOAC 978.10 and AOCS Ba 6a-05, were considered to be not significantly different. However, the “F58 Filter Bag”, which is needed to comply with AOCS Ba 6a-05 is no longer commercially available. The recommended replacement, the “F57 Filter Bag”, which is commercially available has been shown to causes a 10% (relative) low bias. It is doubtful that AOAC 978.10 and AOCS Ba 6a-05, modified for the F57 bag, would be statistically equivalent. Based on lack of availability of the F58 filter bag which is needed to perform AOCS Ba 6a-05, **the committee is recommending AOAC 978.10, Fiber (Crude) in Animal Feed and Pet Food (F.G. Crucible), as the recommended method for crude fiber analysis on DDGS.**





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Table 3 Summary of Results

Results Summary Table			Intralaboratory (SDSU Lab) Results Summary				Interlaboratory (23 labs) Results Summary				
Method	Description	Units	StdDev	CV	Avg Value	Range	StdDev	CV	Avg Value	Range	n
AOAC 934.01	Loss on Drying (Vacuum)	%	0.25	2.34%	10.67		0.75	7.93%	9.50		8 ^a
AOAC 935.29	Loss on Drying (103C/5Hrs)	%	0.15	1.47%	10.17		0.50	5.23%	9.60		7 ^a
NFTA 2.2.2.5	Loss on Drying (105C/3Hrs)	%	0.18	1.82%	9.87		0.44	4.62%	9.50		11 ^b
AOAC 930.15	Loss on Drying (135C/2Hrs)	%	0.19	1.50%	12.69		0.94	8.09%	11.67		17 ^b
AOAC 2001.12	Moisture (Karl Fischer)	%	0.08	0.89%	9.03		3.66	NA	NA		8.08
AOAC 990.03	Crude Protein (Combustion)	%	0.18	0.67%	26.85	0.10	0.43	1.58%	27.05	0.48	17
AOAC 2001.11	Crude Protein (Kjedahl)	%	0.16	0.60%	26.75		0.33	1.23%	26.57		8
AOAC 2003.05	Crude Fat (Ethyl Ether)	%	0.28	3.04%	9.22	4.18	0.84	8.34%	10.02	2.99	7
AOAC 954.02	Fat (Acid Hydrolysis)	%	0.57	4.37%	13.03		0.96	8.07%	11.84		9 ^b
AOAC 945.16	Crude Fat (Pet Ether)	%	0.24	2.71%	8.85		0.27	2.95%	9.13		8 ^a
AOAC 2003.06	Crude Fat (Hexane)	%	0.19	2.11%	9.00		0.48	5.45%	8.85		5
AOAC 978.10	Crude Fiber	%	0.31	4.09%	7.58	0.06	1.26	17.84%	7.06	0.70	6 ^c
AOCS Ba 6a-05	Crude Fiber (Ankom)	%	0.54	7.07%	7.64		0.51	8.10%	6.36		6 ^d

Intralaboratory results are based on averages of 30 test samples analyzed in triplicate for each method at SDSU Olsen Biochemistry Laboratories

Interlaboratory results are based on averages of 5 test samples analyzed in duplicate for each method at various participating laboratories

n = number of labs included in statistical analysis in Phase II

a = two statistical outliers (labs) removed

b = three statistical outliers (labs) removed

c = four statistical outliers (labs) removed

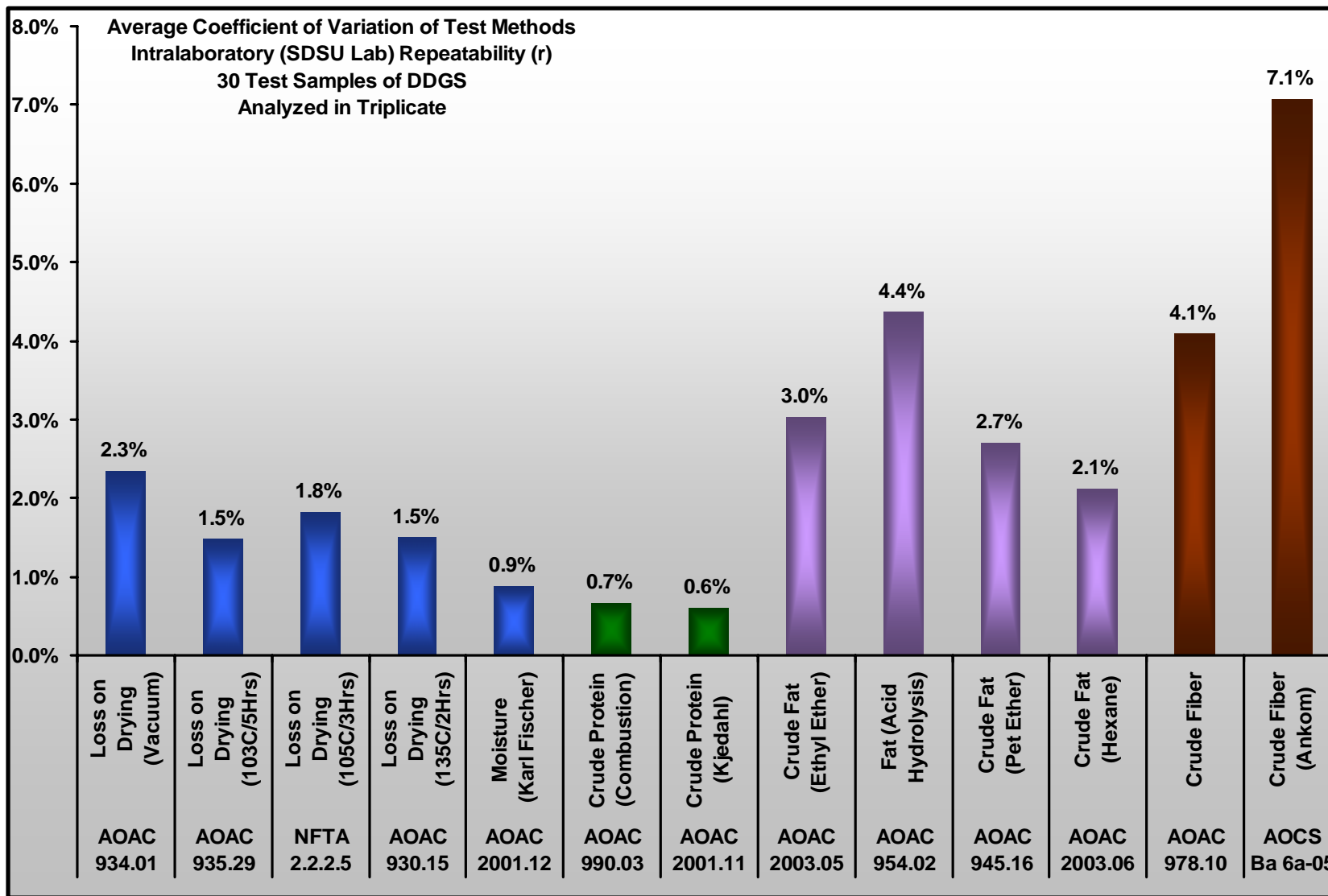
d = one statistical outlier (lab) removed





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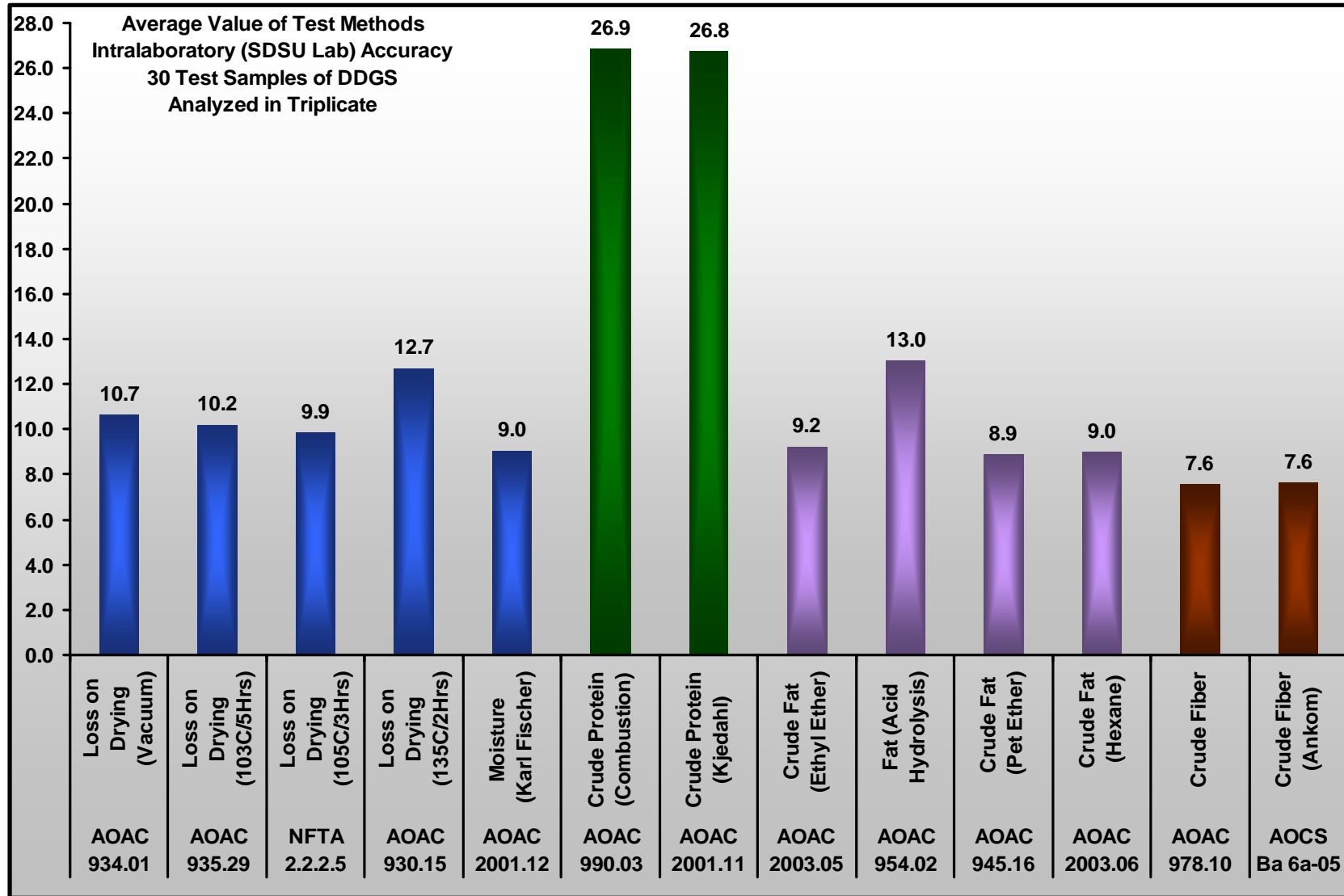
Figure 1





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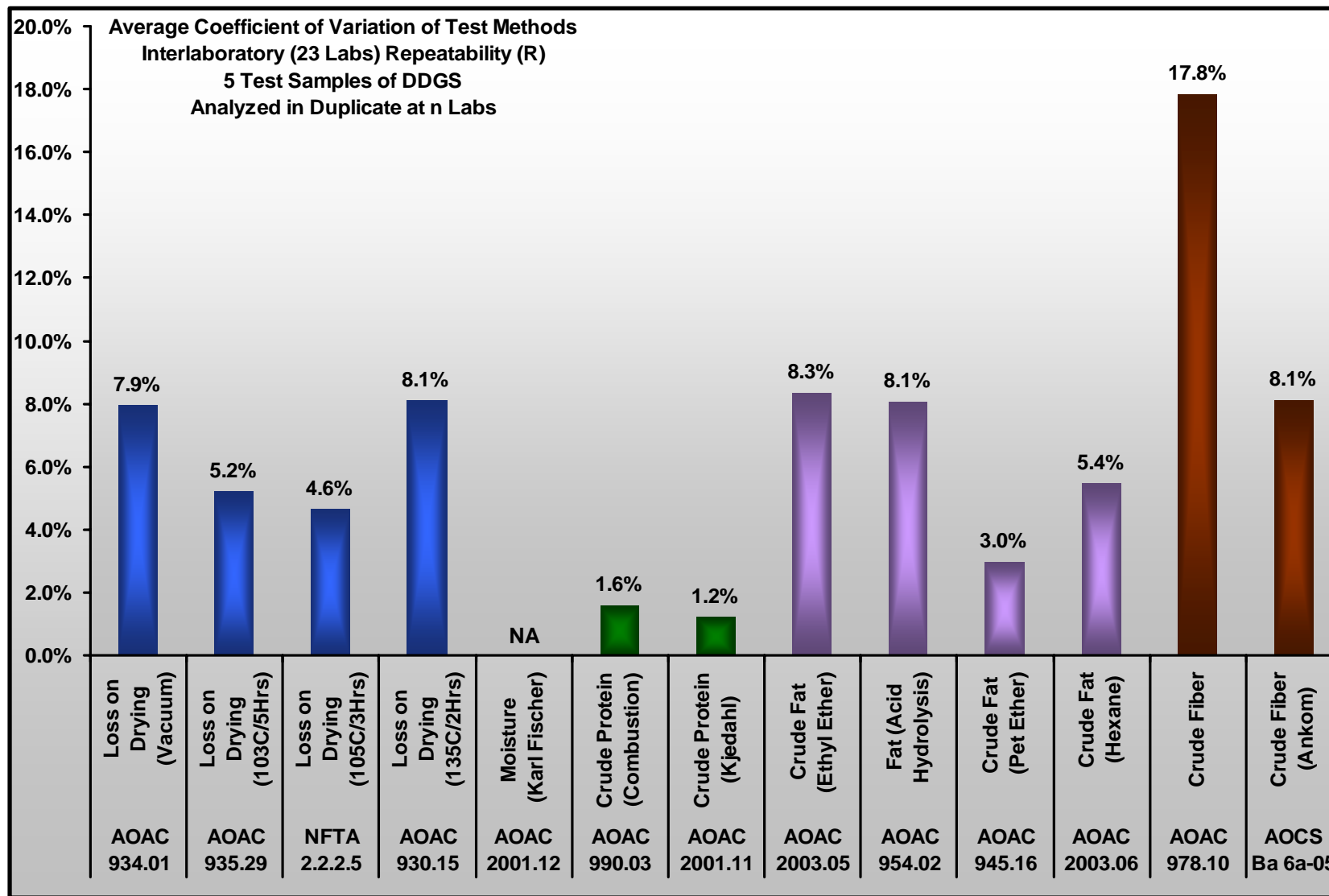
Figure 2





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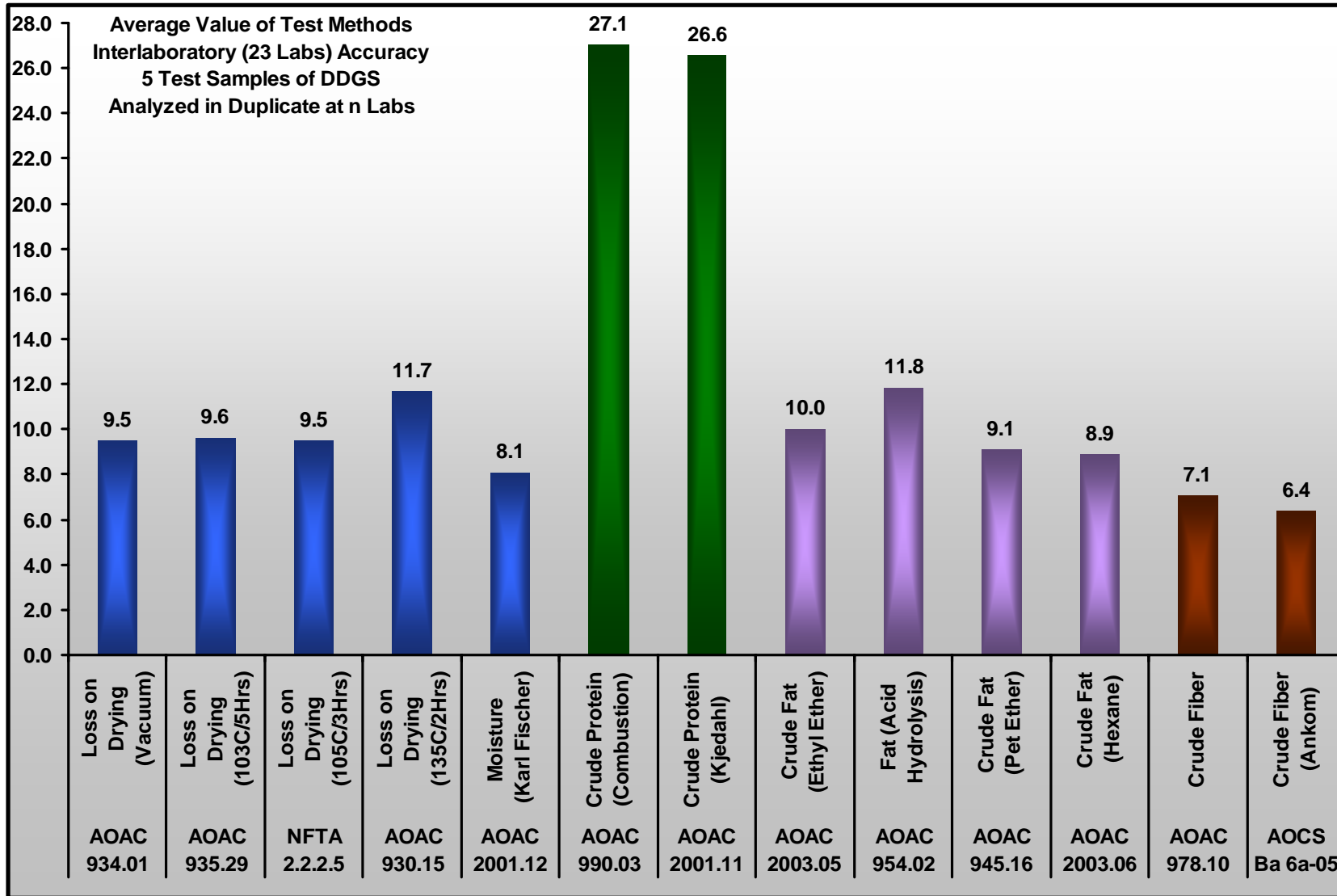
Figure 3





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Figure 4





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Table 4 Final AFIA Committee Method Recommendations

Moisture/Loss on Drying	
NFTA 2.2.2.5	Lab Dry Matter (105 °C / 3 hr)
Crude Protein	
^a AOAC 990.03	Protein (Crude) in Animal Feed - Combustion
^a AOAC 2001.11	Protein (Crude) in Animal Feed and Pet Food (Copper Catalyst)
Crude Fat	
AOAC 945.16	Oil in Cereal Adjuncts (Petroleum Ether)
Crude Fiber	
AOAC 978.10	Fiber (Crude) in Animal Feed and Pet Food (F.G. Crucible)
^a Methods are statistically similar and either is acceptable for use on DDGS	

