Welcome to the

AEIC Composition Working Group

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Fall AEIC Composition Working Group meeting

October 13, 2020 – Virtual

INDUSTRY STATEMENT FOR ANTITRUST COMPLIANCE

There shall be no discussion or activities for the purpose of arriving at any understanding or agreement regarding price, the terms or conditions of sale, distribution, volume of production, territories or customers. There shall be no discussion or activity for the purpose of preventing any person or persons from gaining access to any market or customer for goods or services, nor any agreement or understanding to refrain from purchasing or using any material, equipment, services or supplies. There shall be no discussion or activity that may be construed as forestalling or limiting research and development. We, of course, expect your consideration and full compliance with these guidelines, both while in attendance at this Industry meeting and at all times in your business.

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Composition Working Group Meeting Agenda

- Total Protein Method: Kjeldahl versus Dumas
  - Discussion on available literature and recommendations
- Future topics
  - Fat soluble vitamin multiplexing (A, E, D, K)
  - Omics in risk assessment?

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Dumas and Kjeldahl methods will report different results depending on the non protein nitrogen content of the samples

Kjeldahl

- Use of corrosive / toxic chemicals.
- Low throughput, hours per sample.
- Multiple steps provide potential sources for error.
- Established more than 100 years ago.
- Recovers only organic nitrogen

Dumas

- No corrosive / toxic chemicals
- High throughput, minutes per sample.
- Less prone to human error.
- Gained official recognition in the 90's
- Recovers all nitrogen

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Task at the AEIC 2019 meeting was to evaluate two options

- Conduct laboratory studies
  - Produce uniform set of samples (different crops/matrices)
  - Capture laboratory variability (different labs), operator variability, etc.
  - Make the comparisons of the two methods
  - Come up with conclusions and recommendations
- “White paper” publication based on literature search
  - Lot of information already available
  - Make recommendations based on the literature review
  - Recommendation: “based on available literature, there are differences between methods; both methods are adequate, so choose the best method based on the goal

Oilseed meal showed average bias of +0.09% nitrogen for combustion compared to Kjeldahl attributed to efficiency of combustion method

Comparison of Nitrogen Combustion and $CuSO_4/TiO_2$ Kjeldahl Results

Meal	Combustion (nitrogen %)	Kjeldahl (nitrogen %)	Combustion[a] (bias)
Cottonseed	6.62	6.55	+0.07
Soybean	7.88	7.77	+0.11
Peanut	8.25	8.12	+0.13
Soybean	7.89	7.78	+0.11
Canola	7.21	7.13	+0.08
Safflower	3.34	3.29	+0.05
Safflower	3.32	3.26	+0.06

[a] Average bias +0.09% nitrogen; +0.56% protein, based on factor of 6.25.

Berner and Brown, JOACS, 1994, 71:1291

A cause for the positive bias associated with the nitrogen combustion method is sometimes attributed to ‘nonprotein nitrogen’, possibly from the presence of nitrites (nitrites would not be digested by the Kjeldahl method).

A contribution by nitrites has not been documented. The most likely explanation is that the nitrogen combustion method is more efficient.”

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Cereal grain and oilseeds can be analyzed by combustion method (990.03) used for crude protein in feeds

Sample	Kjeldah	Comb	Kjel-Comb	% Difference
Barley	12.67	12.88	-0.21	1.66
Barley	12.75	12.78	-0.03	0.24
Canola 1	20.89	20.84	0.05	-0.24
Canola 1	20.77	20.85	-0.08	0.39
Canola 2	23.33	23.22	0.11	-0.47
Canola 2	23.09	23.35	-0.26	1.13
Corn	8.83	8.95	-0.12	1.36
Corn	8.75	9	-0.25	2.86
Corn	8.82	8.95	-0.13	1.47
Corn	9.1	9.22	-0.12	1.32
Sorghum	8.73	8.89	-0.16	1.83
Sorghum	8.87	8.9	-0.03	0.34
Soybean 1	35.19	35.16	0.03	-0.09
Soybean 1	35.3	35.39	-0.09	0.25
Soybean 1	34.38	34.7	-0.32	0.93
Soybean 1	35.31	35.06	0.25	-0.71
Soybean 2	41.03	41.24	-0.21	0.51
Soybean 2	40.66	40.9	-0.24	0.59
Sunflower	18.75	18.83	-0.08	0.43
Sunflower	18.34	18.15	0.19	-1.04
Wheat 1	13.36	13.44	-0.08	0.60
Wheat 1	13.17	13.41	-0.24	1.82
Wheat 1	13.12	13.13	-0.01	0.08
Wheat 1	13.54	13.58	-0.04	0.30
Wheat 2	17.18	17.36	-0.18	1.05
Wheat 2	17.14	17.36	-0.22	1.28
Lysine HCl	96.06	95.41	0.65	-0.68
Lysine HCl	96.01	95.47	0.54	-0.56
Nicotinic acid	66.49	70.78	-4.29	6.45
Nicotinic acid	68.62	70.65	-2.03	2.96

780 BICSAK: JOURNAL OF AOAC INTERNATIONAL VOL. 76, NO. 4, 1993

FOOD COMPOSITION

Comparison of Kjeldahl Method for Determination of Crude Protein in Cereal Grains and Oilseeds with Generic Combustion Method: Collaborative Study

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Crops included:
Soy, Canola, Sunflower, Wheat, Barley, Corn, Sorghum

Data across 7 labs

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Increasing the sample size in combustion method improved accuracy

Table IV. Accuracy of Kjel–Foss and Two Combustion Methods for Determining Total Nitrogen Content of Five Pure Chemicals[a]

compd	method I[b]	method II[c]	method III[d]	theor N content or NBS value
NBS std 1572 (citrus leaves)	27.5	29.7	28.2	28.6
NBS std 1573 (tomato leaves)	44.8	52.3	50.7	50.0
NBS std 1577a (bovine liver)	105.3	106.5	107.1	107.0
L-glutamic acid	93.8	96.1	95.6	95.4
EDTA	79.7	95.9	96.1	95.9
acetanilide	101.7	102.5	103.6	103.6
nicotinic acid	110.7	112.1	113.2	113.7
L-Lys·HCl	153.2	152.4	154.0	153.3

[a] Average N results in grams per kilogram ($n = 4$). [b] Kjel–Foss. [c] Low-weight combustion. [d] Macrocombustion.

I = Kjeldahl
II = Dumas – 'x'-mg sample
III = Dumas – 1 g pelletized

Schmitter and Rihs, J. Agric. Food Chem. 1989, 37, 992-994

Results between the two methods depends on matrixes

Table I. Comparison of Kjel-Foss and Two Combustion Methods for Total Nitrogen Determination[a]

product	method			difference		
	I[b]	II[c]	III[d]	I–II	I–III	II–III
straw 1	5.6	7.1	5.3	−1.5	0.3	1.8
straw 2	6.2	6.1	6.5	0.1	−0.3	−0.4
corn silage 1	11.2	12.1	11.4	−0.9	−0.2	0.7
porc soup 1	11.8	12.0	11.7	−0.2	0.1	0.3
hay 1	12.6	12.7	12.9	−0.1	−0.3	−0.2
corn silage 2	13.1	14.5	13.1	−1.4	0.0	1.4
corn grain 1	13.4	14.0	13.5	−0.6	−0.1	0.5
corn grain 2	13.9	14.3	13.8	−0.4	0.1	0.5
barley 1	15.6	15.7	14.6	−0.1	1.0	1.1
barley 2	16.9	18.1	17.2	−1.2	−0.3	0.9
oats 1	17.4	18.0	17.2	−0.6	0.2	0.8
grass silage 1	17.3	18.9	17.8	−1.6	−0.5	1.1
oats 2	18.1	18.7	17.9	−0.6	0.2	0.8
triticale 1	18.3	18.7	18.0	−0.4	0.3	0.7
wheat 1	19.5	20.0	19.7	−0.5	−0.2	0.3
hay 2	19.8	21.4	20.7	−1.6	−0.9	0.7
dried grass 1	22.2	22.8	22.0	−0.6	0.2	0.8
grass silage 2	24.0	24.5	24.9	−0.5	−0.9	−0.4
cow premix 1	26.6	28.2	26.9	−1.6	−0.3	1.3
NBS citrus leaves	27.5	29.7	28.2	−2.2	−0.7	1.5
dried grass 2	34.0	33.9	34.7	0.1	−0.7	−0.8
porc soup 2	34.1	33.6	34.2	0.5	−0.1	−0.6
coconut meal	34.8	36.9	36.1	−2.1	−1.3	0.8

[a] N results in grams per kilogram (nitrogen range: 5–40 g/kg). [b] Kjel–Foss. [c] Low-weight combustion. [d] Macrocombustion.

Table II. Comparison of Kjel–Foss and Two Combustion Methods for Total Nitrogen Determination[a]

product	method			difference		
	I[b]	II[c]	III[d]	I–II	I–III	II–III
milk powder	42.7	43.2	44.1	−0.5	−1.4	−0.9
bone meal 1	43.4	44.4	42.1	−1.0	1.3	1.3
NBS tomato leaves	44.8	52.3	50.7	−7.5	−5.3	1.6
rapeseed 1	54.7	57.0	57.2	−2.3	−2.5	−0.2
protein conc 1	57.9	60.9	60.0	−3.0	−2.1	0.9
protein conc 2	62.8	62.6	63.2	0.2	−0.4	−0.6
bone meal 2	63.7	63.3	63.7	0.4	0.0	−0.4
soybean meal 1	64.4	64.3	65.4	0.1	−1.0	−1.1
yeast 1	67.2	68.3	69.6	−1.1	−2.4	−1.3
soybean meal 2	67.9	69.9	69.9	−2.0	−2.0	0.0
peanut meal	82.4	84.0	85.0	−1.6	−2.6	−1.0
meat meal 1	93.7	92.4	93.6	1.3	0.1	−1.2
meat meal 2	96.8	99.2	99.6	−2.4	−2.8	−0.4
fish meal 1	100.0	99.3	99.9	0.7	0.1	−0.6
NBS bovine liver	105.3	106.5	107.1	−1.2	−1.8	−0.6
fish meal 2	117.0	117.6	119.7	−0.6	−2.7	−2.1
wheat gluten 1	117.6	117.5	117.1	0.1	0.5	0.4
wheat gluten 2	134.0	135.3	134.0	−1.3	0.0	1.3
greaves 1	138.9	140.8	139.5	−1.9	−0.6	1.3
greaves 2	139.5	140.6	141.8	−1.1	−2.3	−1.2

[a] N results in grams per kilogram (nitrogen range: 40–140 g/kg). [b] Kjel–Foss. [c] Low-weight combustion. [d] Macrocombustion.

I = Kjeldahl
II = Dumas – ‘x’-mg sample
III = Dumas – 1 g pelletized

Schmitter and Rihs, J. Agric. Food Chem. 1989, 37, 992-994

Canadian Grain Commission replaced Kjeldahl with combustion for oilseed surveys

- Nitrogen analyses for canola seed, flaxseed, sunflower seed, mustard seed and soybeans
- The nitrogen analyzer gave significantly higher values than the Kjeldahl method, resulting in a correction of low values in the GRL Kjeldahl, caused by the inability to use mercury as catalyst.
- Coefficient of variation for combustion method (0.03%) was only slightly higher than for the Kjeldahl method (0.024%).
- Slightly higher errors in the combustion analysis may be due to the relatively small sample size (125 mg) used in our analysis. Because of the small sample size, it is especially important to obtain a fine grind of the sample. Oilseeds such as cottonseed and sunflower seed, which have fibrous hulls, may cause repeatability problems due to the difficulties in obtaining finely ground samples.
- Except for sunflower seed, higher values with Dumas.

Duan, J. JAOCS (1994) 71, 1069-1072

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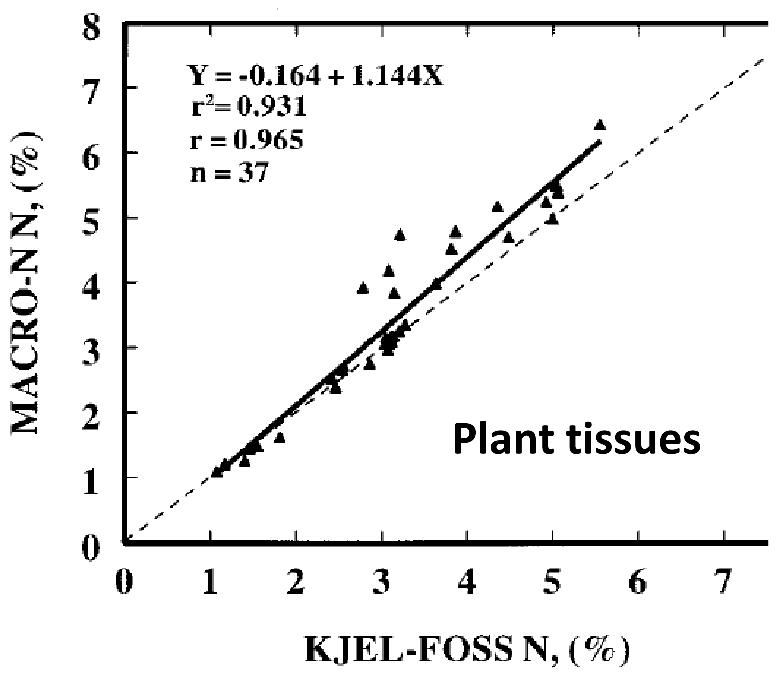
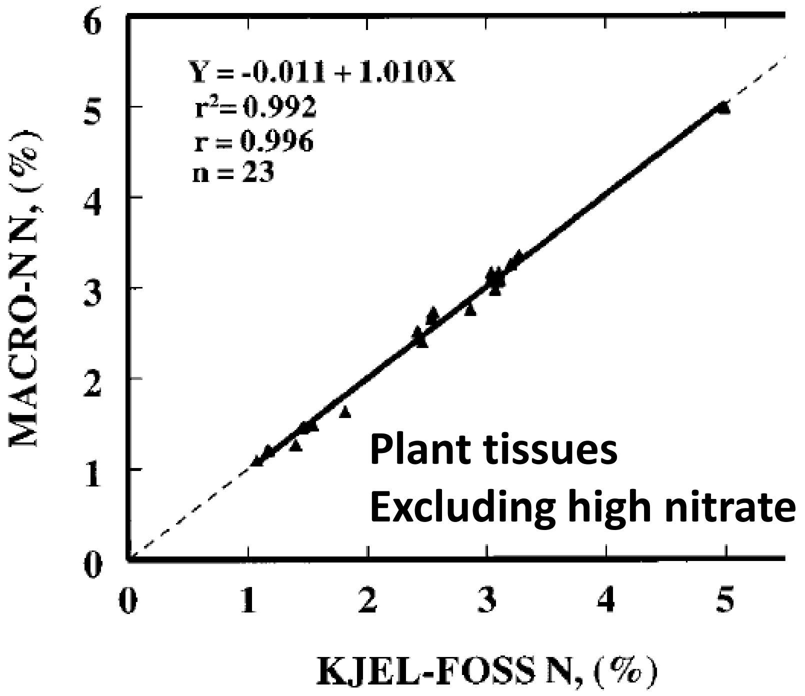
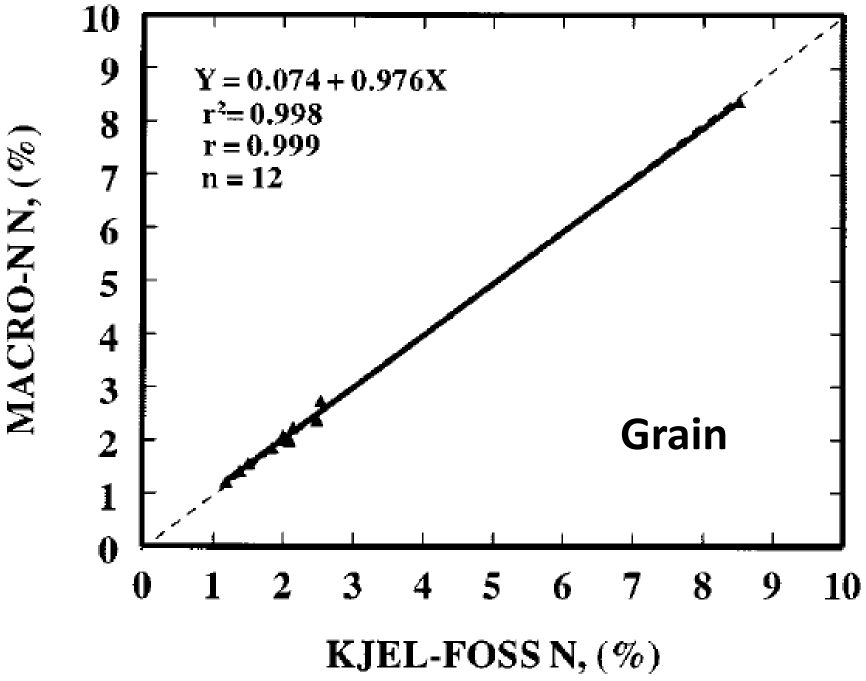
Correlation between Dumas (Macro-N) and Kjeldahl (KjelFoss) methods is matrix dependant







Watson and Galliher, Communications in Soil Science and Plant Analysis (2001) 32: 13-14, 2007-2019

Good correlation between both methods in animal feed

Table 7
Comparison of KM and CM accuracy

	Corn	Barley	LU2	SP	DF	SOP	MP	FP1
CP% KM, $\bar{x} \pm s$ ($n = 3$)	7.28 ± 0.09	10.6 ± 0.08	17.9 ± 0.1	26.0 ± 0.1	27.1 ± 0.2	38.8 ± 0.4	51.8 ± 0.3	71.6 ± 0.2
CP% CM, $\bar{x} \pm s$ ($n = 5$)	7.6 ± 0.3	10.54 ± 0.06	18.2 ± 0.2	26.0 ± 0.3	27.1 ± 0.2	38.9 ± 0.5	51.8 ± 0.4	71.6 ± 0.4
Difference (%)	+3.7	−0.9	+1.7	−0.4	+0.2	+ 0.3	0.0	0.0
P (t-test)	0.12	0.11	0.14	0.76	0.75	0.70	0.94	0.94

LU2, lupines; SP, sunflower powder; DF, ‘Delicias’ foodstuff; SOP, soya powder; MP, meat powder; FP1, fish powder.

Marco et al, Talanta 57 (2002) 1019-1026

A regression equation was developed to convert Dumas N to Kjeldahl N for soybean products

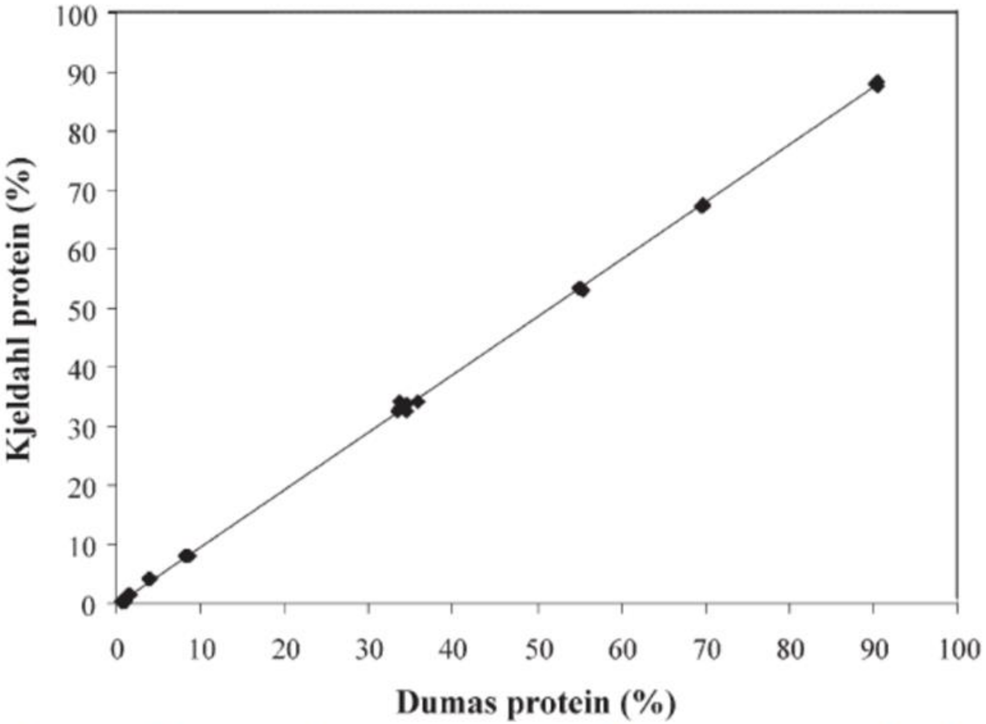


FIG. 1. Relationship between protein values (%) obtained by the Kjeldahl and Dumas methods ($y = -0.00536 + 0.97188x$, $R^2 = 0.9997$).

TABLE 3
Protein Contents (% as is) Obtained by the Dumas and Kjeldahl Methods[a]

Soy product	Mean Kjeldahl	Mean Dumas	Ratio K/N[b]
Soy protein isolate	88.09[a]	90.33[b]	0.975
Soy protein concentrate	67.35[a]	69.76[b]	0.965
Defatted soy flakes	53.43[a]	55.23[b]	0.967
Soy protein isolate curd	34.03[a]	34.45[b]	0.987
Soybeans	32.88[a]	33.79[b]	0.973
Tofu	7.92[a]	8.35[a]	0.948
Protein extract	4.11[a]	3.99[a]	1.030
Soymilk	1.49[a]	1.55[a]	0.961
Whey	0.50[a]	0.76[a]	0.657

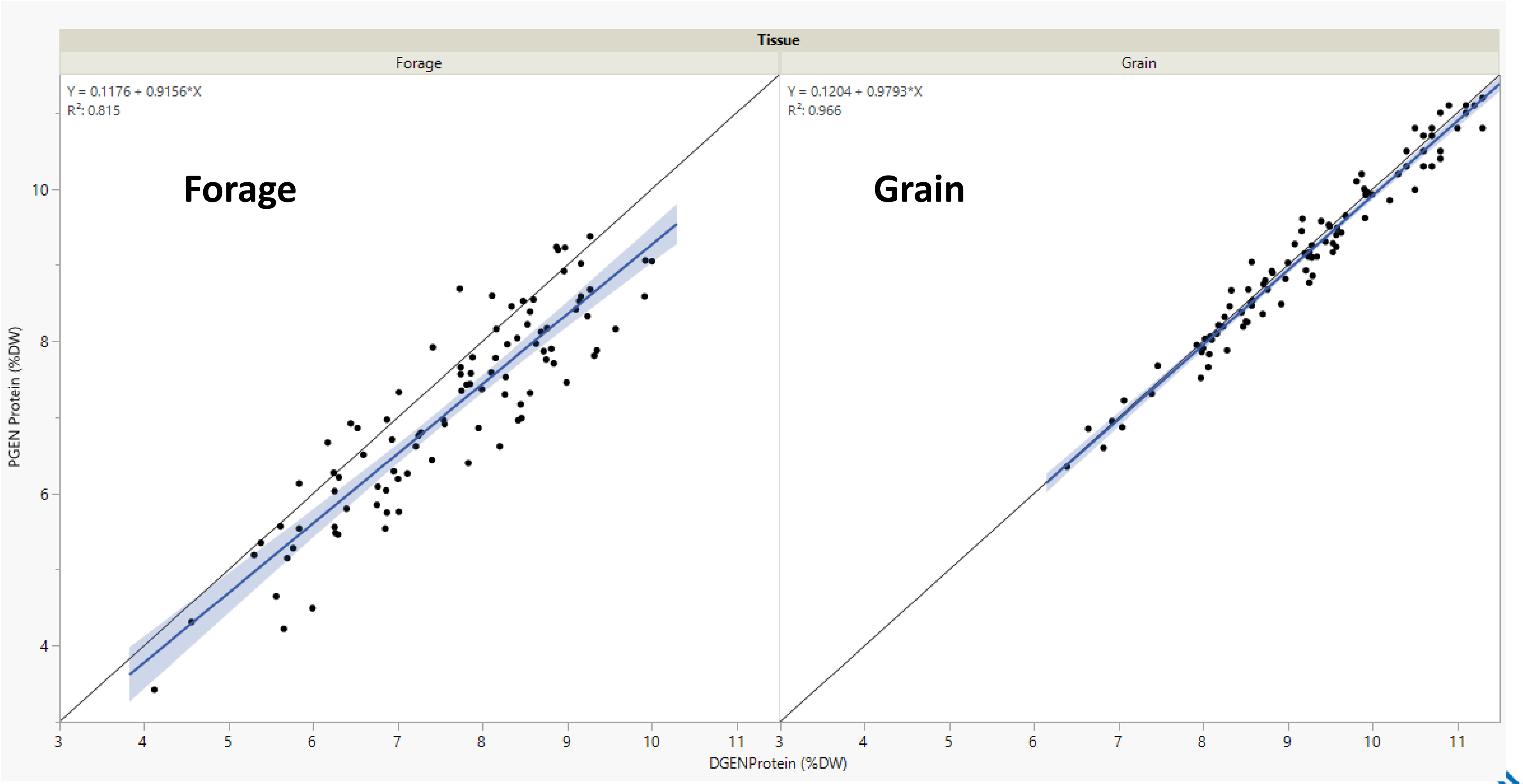
[a]Means are followed by SD. Mean protein contents for the Kjeldahl and Dumas methods in the same row followed by different letters are significantly different ($P < 0.05$).
[b]The K/N value is the ratio between the mean Kjeldahl value and the mean Dumas value.

Jung et al. JAOCS 2003, 80, 1169-1173

Literature available

- Comparison data for Kjeldahl vs combustion are available for
  - Oilseeds
  - Cereal grain
  - Oilseeds meal
  - Animal feed
- No data on forage samples from corn or soybean

Comparison of protein levels in maize grain and forage using Kjeldahl vs. Combustion (Data from Syngenta)



96 Samples
8 Locations
6 Hybrids

X-Y correlation of DGEN values (x-axis) and PGEN values (y-axis) by tissue type. Blue line and shaded area indicate line of fit with confidence region. Black line designates perfect correlation (y=x).

Comparison of protein levels in maize grain and forage and canola seed using Kjeldahl vs. Combustion (Data from EPL)

Matrix	Sample Weight~	Kjeldaghl	Sample Weight~	Dumas	% Difference
Maize Grain	1.25g	7.23	1g	7.51	3.87
Maize Grain	1.25g	7.29	1g	7.47	2.54
Maize Grain	1.25g	6.64	1g	7.42	11.76
Maize Grain	1.25g	7.25	1g	7.55	4.08
Maize Grain	1.25g	7.24	1g	7.59	4.83
Maize Grain	1.25g	7.33	1g	7.42	1.19
Maize Grain	1.25g	7.22	1g	7.41	2.63
Maize Grain	1.25g	7.38	1g	7.64	3.55
Maize Grain	1.25g	7.24	1g	7.47	3.13
Maize Grain	1.25g	7.20	1g	7.58	5.22
Maize Forage	1.25g	8.16	1g	8.41	3.08
Maize Forage	1.25g	8.09	1g	8.52	5.35
Maize Forage	1.25g	8.00	1g	8.62	7.82
Maize Forage	1.25g	8.07	1g	8.50	5.37
Maize Forage	1.25g	8.04	1g	8.51	5.83
Maize Forage	1.25g	8.04	1g	8.51	5.85
Maize Forage	1.25g	7.91	1g	8.61	8.90
Maize Forage	1.25g	8.22	1g	8.60	4.61
Maize Forage	1.25g	8.20	1g	8.46	3.12
Maize Forage	1.25g	8.00	1g	8.42	5.21

Matrix	Sample Weight~	Kjeldahl	Sample Weight~	Dumas	% Difference
Canola Seed	0.5g	25.126	1g	25.7	2.28
Canola Seed	0.5g	24.680	1g	25.7	4.13
Canola Seed	0.5g	24.972	1g	25.7	2.92
Canola Seed	0.5g	25.415	1g	25.7	1.12
Canola Seed	0.5g	25.135	1g	25.7	2.25
Canola Seed	0.5g	24.733	1g	25.8	4.31
Canola Seed	0.5g	24.876	1g	25.7	3.31
Canola Seed	0.5g	25.242	1g	25.7	1.81
Canola Seed	0.5g	25.198	1g	25.7	1.99
Canola Seed	0.5g	25.629	1g	25.7	0.28

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Summary and recommendations

- Difficult to generalize a conversion factor for nitrogen values analyzed by Kjeldahl versus combustion
- Differences in methods will depend on matrix, crop, tissue, and growing conditions.
- Both methods can be used when the differences is negligible, but the method used has to be clearly stated.
- From Ag Biotech prospective, there is an opportunity to generate and publish multi-lab data from seeds/grain, forage, meal and feed samples and investigate the origin of differences between the two methods: inorganic N or efficiency
    - Need to secure funding for such experiment.

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