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A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory

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Abstract

The Dumas combustion procedure using Leco's CNS 2000 analyzer has been shown to give nitrogen values comparable to those obtained with the Kjeldahl procedure for soil and plant products (slightly higher for most samples). These procedures were compared for a range of samples more typical for an animal nutrition laboratory (feed, excreta, carcass, egg yolk, milk and urine). For the 36 samples compared, the Kjeldahl procedure gave N values slightly lower than the Dumas procedure: $N_{Kjeldahl} = 0.0103 + 0.9855 \times N_{CNS}$. The coefficient of variation for standard AAFCO broiler and cattle feeds (measured repeatedly over an 18-month period) were 2.23 (n=90) and 2.12 g/100 g (n=177), respectively. It was concluded that the Dumas combustion procedure is capable of replacing the Kjeldahl procedure for routine animal nutrition laboratory N analyses. The Dumas procedure has the advantages of using fewer strong reactants, requiring less labor, and uses a more efficient temperature to release the nitrogen from the samples than the Kjeldahl procedure.

Keywords: Nitrogen; Kjeldahl; Dumas; Protein; Combustion

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1. Introduction

Because of increasing government regulations and the increase in cost for disposal of hazardous materials, the Dumas combustion technique (Dumas, 1826 as cited by Buckee, 1994) for total nitrogen has received much attention in recent years. McGeehan (1988) reconfirmed that the Kjeldahl nitrogen value for materials containing nitrates were lower than values obtained using combustion techniques. In general, the combustion process gave slightly higher nitrogen values than wet oxidation techniques when analyzing plant material (Jones, 1992; Buckee, 1994; Simonne et al., 1995). Soil nitrogen values obtained by the Kjeldahl analysis were lower than those obtained with the combustion process although Wang (1993) concluded that the Kjeldahl procedure gave better precision and accuracy than the combustion technique on finely ground samples. It appears that more research data are needed on soils to determine which procedure is the most accurate.

The Dumas combustion method has been adopted as a alternative technique for total nitrogen by the feed industry (Sweeney, 1989) and as the official reference method by the Brewing industry (Buckee, 1994). The next few years will probably be a transition period from the Kjeldahl procedure which utilizes many hazardous materials to the environmentally friendly combustion technique. The purpose of the experiments described here were to compare the Kjeldahl and Dumas nitrogen procedures using a variety of samples typical of an animal nutrition analytical laboratory.

2. Materials and methods

2.1. Samples analyzed

One hundred and twenty-six samples from animal nutrition feeding trials with a broad range of nitrogen contents were randomly chosen from samples submitted to our laboratory (8–180 g kg^{-1} N; Table 1). The feed and excreta samples were milled to pass a 1.0 mm screen (Wiley Mill; Arthur H. Thomas, Philadelphia, PA, USA). Excreta samples were dried at 70°C overnight in a forced air oven, after which they were allowed to air equilibrate. Milk samples were weighed into a nitrogen-free plastic film (Saran Wrap, Dow, Indianapolis, Indiana, USA). Urine samples were pipetted directly into the digestion flask. Meat samples were ground to pass a 5 mm screen (Hobart meat grinder; Hobart, Troy, OH, USA). Egg yolks were freeze dried (Virtis, Gardiner, NY, USA), then ground with a mortar and pestle.

2.2. Accuracy

Twelve nitrogenous compounds (reagent grade) were dried at 105°C for 2 h and stored in a desiccator until analyzed (Sigma, St. Louis, Missouri). Sample compounds in the range from approximately 9–47 g kg^{-1} nitrogen were used in this experiment (alanine, arginine, glycine, histidine, lysine, urea, methionine, aspartic acid, cystine, nicotinic acid, tryptophan, ethylenediaminetetra acetic acid). Samples were analyzed in duplicate. Theoretical nitrogen values were calculated as a mass percentage from the molecular formula for each compound.

Table 1
Descriptive statistics of data used to compare Kjeldahl and Dumas methods for nitrogen analysis

Sample	Method	N	Mean (g kg^{-1} N)	Min. (g kg^{-1} N)	Max. (g kg^{-1} N)	Standard Deviation (SD)	CV (SD/$\bar{x}$ 0.01)
Feces+excreta [a]	Kjeldahl	20	100.0	50.7	170.6	45.2	451.8
	Dumas	20	101.6	50.6	173.5	45.8	450.5
Feed [b]	Kjeldahl	41	41.9	13.1	101.7	30.0	716.1
	Dumas	41	42.6	13.1	101.4	30.1	706.3
Milk [c]	Kjeldahl	9	8.7	8.2	10.0	0.6	65.7
	Dumas	9	8.7	8.1	10.3	0.7	80.6
Urine [d]	Kjeldahl	3	42.1	39.5	43.8	2.3	53.9
	Dumas	3	42.7	40.5	44.1	2.0	46.4
Egg yolks	Kjeldahl	9	26.7	26.6	27.1	0.2	6.2
	Dumas	9	27.3	26.9	27.8	0.3	10.5
Carcass [e]	Kjeldahl	44	29.6	25.8	32.5	1.4	48.1
	Dumas	44	29.7	25.4	33.3	1.8	60.6

[a] Eight poultry excreta and 12 bovine feces.
[b] Thirty-one broiler fed and 10 bovine feed.
[c] Porcine milk.
[d] Rat urine.
[e] Poultry carcass.

2.3. Precision

Two quality control feed samples were obtained from The Association of American Feed Control Officials (AAFCO feed check sample, University of Kentucky, Lexington, KY, USA). The broiler feed (approximately 3.18 g kg^{-1} N) and cattle feed (approximately 6.77 g kg^{-1} N) were analyzed repeatedly by the Dumas procedure over an 18-month period, as time permitted.

2.4. Kjeldahl procedure (AOAC, 1990)

The sample sizes used in the Kjeldahl procedure were dried feces or excreta, 0.5 g; feed, 1.0 g; urine, 5 ml; carcass, 1.0 g; milk, 1.5 g; and freeze dried egg yolk, 0.5 g. Samples were weighed and transferred into the Kjeldahl digestion flask containing 15 g of catalyst (96% K_2SO_4/4% $CuSO_4.5H_20$) and 25 ml of concentrated H_2SO_4. After $2\frac{1}{2}$ h of digestion in a unit with electrical heat and fume removal (Labonco, Kansas City, MO, USA) and cooling to room temperature, 400 ml of distilled water, 100 ml of 50% NaOH and 0.25 g Zn were added to each flask. By distillation, ammonium hydroxide was trapped as ammonium borate in a 4% boric acid solution (4 g of boric acid in 100 ml deionized water (w/v)). Total nitrogen was determined by titration with standardized HCL to a mixed indicator endpoint (0.1 g/100 ml bromocresol green and 0.1 g/100 ml methyl red in 95% ethanol).

2.5. Dumas combustion procedure Leco CNS 2000

Several sample weights for each sample type were analyzed to determine optimum sample size when using the Dumas combustion method (Leco, St. Joseph, MI, USA).

Optimum sample sizes were: dried feces or excreta, 0.2 g; feed, 0.2 g; urine, 0.1–0.5 ml; carcass, 0.5 g; milk, 1.0 ml; and freeze dried egg yolk, 0.2 g. Samples were weighed into a porcelain sample holder (boat) for introduction into the combustion chamber (1300°C) utilizing an automated sample loader (CNS 2000, Operation Manual, Leco, St. Joseph, MI, USA). The combustion process converts covalently bound nitrogen into nitrogen gas (N_2). The N_2 is quantitated by passing the gas through a conductivity cell.

2.6. Statistical analyses

Statistical analyses were performed using PROC GLM (general linear models) of the Statistical Analysis System (SAS, 1988). Regression analyses were performed to characterize the effect of the Dumas method as a function of the Kjeldahl method for different sample types. To evaluate the repeatability of the Dumas method, sources of variation in the analysis of variance included the effects for feed sources, replication over days, and the interaction between feed source and day.

3. Results and discussion

The agreement between the Kjeldahl and Dumas methods for typical samples in our laboratory has been excellent for a broad range of samples between about 8 and 180 g kg^{-1} nitrogen (Table 1 and Fig. 1). In all cases the Dumas combustion procedure

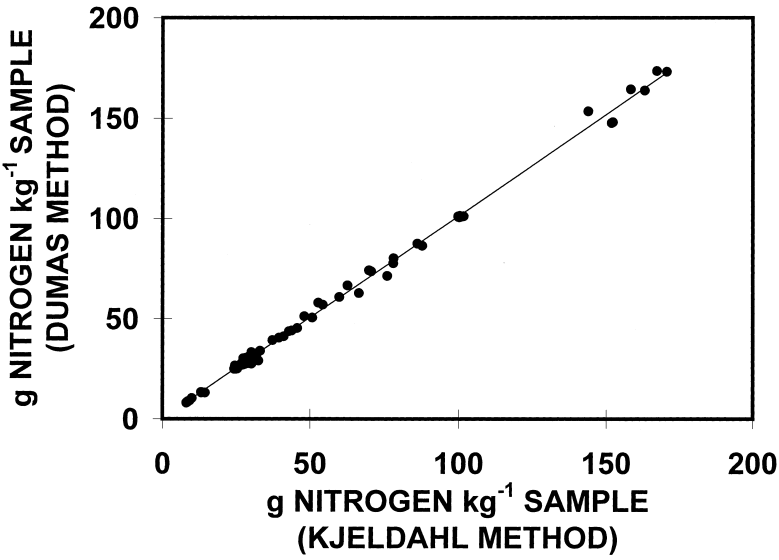

Fig. 1. The nitrogen content of feces, excreta, feed, urine, carcass, milk and freeze dried egg yolk as determined by the Kjeldahl and Dumas methods. Samples were analyzed in duplicate.

Table 2
Regression analysis for predicting Kjeldahl nitrogen as a function of Dumas nitrogen (Kjeldahl=B_0+B_1 Dumas)

	N	B_o (The intercept)		B_1 (The slope)		R^2
		Estimate±SE [a]	Pr>|T| [b]	Estimate±SE	Pr>|T|	
Overall	126	0.0103±0.0253	0.6857	0.9855±0.0044	<0.0001	0.9975
Feces	20	0.0080±0.2178	0.9711	0.9839±0.0196	<0.0001	0.9929
Feed	41	−0.0567±0.0233	0.0197	0.9987±0.0045	<0.0001	0.9992
Milk	9	0.1673±0.0330	0.0015	0.8146±0.0379	<0.0001	0.9851
Urine	3	−0.6507±0.5441	0.4434	1.1353±0.1271	0.0710	0.9876
Egg yolks	8	1.6743±0.4595	0.0082	0.3702 [c]±0.1680	0.0634	0.4095
Carcass	44	1.5152±0.2860	<0.0001	0.4858 [c]±0.0962	0.0001	0.3778

[a] Standard Error of Estimate.
[b] Probability that the coefficient is significantly different from zero.
[c] The coefficient is significantly different from 1.0 at P<0.05.

results have been very similar to those from the more laborious and hazardous chemical consuming Kjeldahl procedure.

Regression analysis of the overall comparison of data from 126 samples clearly indicates that the results of the two procedures are comparable (Table 2). For all the data, the intercept of the line describing Kjeldahl values as a function of Dumas values was clearly not different from zero and the slope is not different from unity. Results of either procedure can be used to predict results of the other with a very high degree of accuracy. Still it is very interesting that for two classes of samples, egg yolks and ground carcasses, the slopes appear to be significantly different from unity using a one-tailed t-test and 0.05 level of probability. These samples are in the low end of the range of nitrogen levels studied, but still appear to be in the linear range (Fig. 1). Differences in the intercept compensate for differences in the slope so that the samples are predicted very well using the overall line. Results in our nutrition laboratory are in agreement with results from agronomy samples (Jones, 1992); brewing products (Buckee, 1994); and horticulture samples (Simonne et al., 1995). All reported slightly higher values overall with the Leco combustion method.

The coefficients of variation with the Dumas procedure over all broiler and cattle feed individual observations were only about 2% (Table 3). The significant effect of day in the analysis of variance table (Table 3) comes from days when extremely high and low values were obtained. The highest and lowest days for the broiler feed were 3.06±0.03 (n=2 samples) and 3.24 (n=1); and for the cattle feed were 6.50±0.03 (n=9) and 6.98±0.07 (n=2). Such aberrant results would have been called for re-testing by our quality control program. The AAFCO standards are normally analyzed each day. When the standards are above or below expectations, that entire days samples are repeated. But the deviations from expectations observed in Table 3 may also have been due to random sampling errors. Mixed feeds are by nature heterogenous substances and even with great care in sampling methods, some variation will occur. The variability observed in Table 3 is not at all unusual for samples analyzed repeatedly over an extended period of time.

Table 3
Descriptive statistics and analysis of variance of data used to compare results of the Dumas nitrogen analysis using the Leco CNS 2000 analyzer to analyze a reference cattle feed and a broiler feed over time

Sample	N	Mean (g kg^{-1} N)	Min. (g kg^{-1} N)	Max. (g kg^{-1} N)	Standard Deviation	CV (%)
Broiler feed [a]	90	31.58	29.50	32.76	0.72	2.28
Cattle feed [b]	177	67.55	63.77	71.88	1.46	2.16
ANOVA						
Source	df [c]	Pr>*F*				
Day	73	0.0001				
Feed	1	0.0001				
Feed×Day	1	0.4514				
Error	191					

[a] One to 9 replicate samples/day on each of 28 days, mean=3.21 replicates/day.
[b] One to 9 replicate samples/day on each of 48 days, mean=3.69 replicates/day.
[c] Degrees of freedom.

Table 4
Recovery of nitrogen using the Kjeldahl and Dumas methods

Material [a]	Theoretical [b] (g kg^{-1} N)	Dumas (g kg^{-1} N)	Kjeldahl (g kg^{-1} N)	Dumas recovery [c]	Kjeldahl recovery [c]
EDTA	95.9	95.8	94.6	0.9990	0.9864
Tryptophan	137.2	137.6	134.3	1.0029	0.9789
Nicotinic acid	113.8	114.1	87.4	1.0026	0.7680
Cystine	116.6	116.0	112.6	0.9949	0.9657
Aspartic acid	105.2	105.4	103.4	1.0019	0.9829
Methionine	93.9	93.4	92.5	0.9947	0.9851
Urea	466.5	471.3	456.0	1.0103	0.9775

[a] Duplicate determinations were performed on each nitrogenous compound.
[b] Theoretical nitrogen values were calculated from the molecular formula for each compound.
[c] Recovery=measured/theoretical.

Table 4 shows the recovery of various nitrogenous materials using the Dumas and the Kjeldahl nitrogen procedures. The samples were analyzed in duplicate. These results show very good recoveries for both methods except for nicotinic acid by the Kjeldahl procedure. Isaac and Johnson (1976) attributed their low recovery of nicotinic acid to the inability of the low temperature (332°C) of the block digester to break the double bonded ring structure. The recoveries for all materials tested including nicotinic acid appeared to be higher for the Dumas combustion technique (Table 4). There was excellent agreement between the Dumas and Kjeldahl procedures for the reagent grade compounds (R^2=0.995956, Fig. 2). The obvious exception was for nicotinic acid where the Dumas method was closer to the predicted N composition. The Dumas method gave slightly higher values than the Kjeldahl procedure but the means were not significantly different.

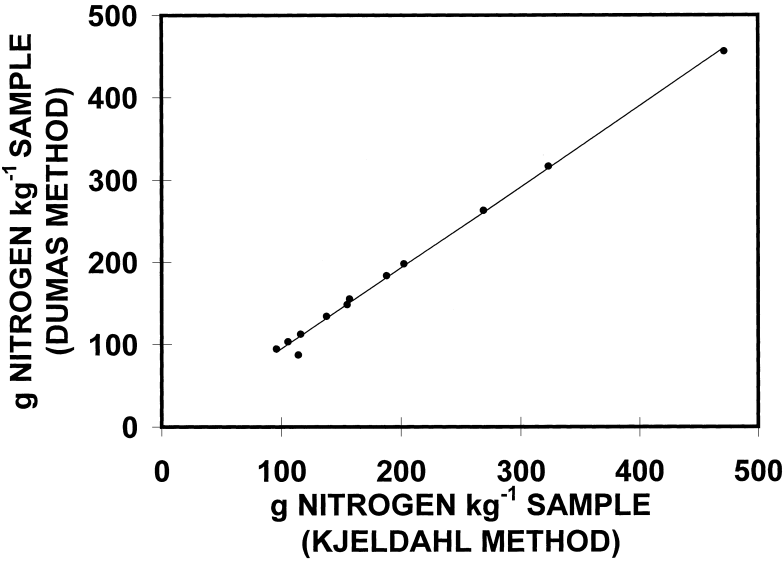

Fig. 2. Nitrogen content of twelve reagent grade compounds as determined by the Kjeldahl and Dumas methods (alanine, arginine, glycine, histidine, lysine, urea, methionine, aspartic acid, cystine, nicotinic acid, tryptophan, and ethylenediamine tetraacetic acid). Compounds were obtained from Sigma, St. Louis, Missouri. Compounds were chosen from a range of values from 9 to 47 g nitrogen kg^{-1} sample from materials of known nitrogen content.

This is probably the result of the more efficient 1300°C temperature used to combust the material rather than the presence of nitrates as suggested by McGeehan (1988).

In conclusion, the Dumas combustion procedure for the measurement of nitrogen in typical samples has been shown to be reasonably accurate and precise. The Dumas procedure, as implemented in the Leco CNS 2000 may replace the Kjeldahl procedure for routine analyses in an animal nutrition laboratory.

References

Association of Official Analytical Chemist 1990. Official Methods of Analysis, 15th edn. Association of Official Analytical Chemist, Washington D.C.

Buckee, G.K., 1994. Determination of total nitrogen in barley, malt and beer by Kjeldahl procedures and the Dumas Combustion Method. J. Inst. Brewing 100, 57–64.

Isaac, R.A., Johnson, W.C., 1976. Determination of total nitrogen in plant tissue, using a block digester. Journal of the AOAC 59, 98–100.

Jones, J.B., 1992. Methods of nitrogen determination in soils and plant tissue. Commun. Soil Sci. Plant Anal. 23, 17–20.

McGeehan, S.L., Naylor, D.V., 1988. Automated instrumental analysis of carbon and nitrogen in plants and soil samples. Commun. In Soil Sci. Plant Anal. 19, 493–505.

Simonne, E.H., McCrimmon, J.N., Mills, H.A., Scoggins-Mantero, H.L., Cresman III, C.P., 1995. Adjustments of sufficiency ranges of selected ornamentals and turfgrasses for assessing nitrogen status with Dumas-N data. Commun. Soil Sci. Plant Anal. 26, 2243–2251.

Statistical Analysis Systems Institute, 1988. SAS/STAT Users Guide, Release 6.03 ed., SAS Institute, Inc., Cary, NC, p. 1028.

Sweeney, J., 1989. Generic combustion method for determination of crude protein in feeds collaborative study. Journal of the Association of Official Analytical Chemist 72, 770.

Wang, D., Snyder, M., Michael, C., Bormannn, H.F., 1993. Potential errors in measuring nitrogen content of soils low in nitrogen. Soil Sci. Soc. Am. J. 57, 1533–1536.