AGRICULTURAL MATERIALS

Repeatability and Reproducibility of Determination of the Nitrogen Content of Fishmeal by the Combustion (Dumas) Method and Comparison with the Kjeldahl Method: Interlaboratory Study

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Ten fishmeal samples (hidden duplicates of 4 meals plus 2 high-protein meals as a Youden pair), tryptophan, and nicotinic acid were analyzed by 18 laboratories using the Dumas method. Thirteen of the laboratories also analyzed the same 12 samples using their current Kjeldahl method. Recoveries (± s_R) of tryptophan and nicotinic acid were 99.3 ± 1.04 and 98.8 ± 2.11% by Dumas and 97.1 ± 3.03 and 74.6 ± 26.76% by Kjeldahl. The Dumas method gave significantly greater values ($P < 0.001$) than the Kjeldahl method. For fishmeals, Kjeldahl N = 0.989 of Dumas N ($P < 0.001$). A similar proportionate difference (0.984 of Dumas N) was observed with tryptophan. Most laboratories failed to determine nicotinic acid correctly by Kjeldahl. For fishmeals, the relative standard deviations for repeatability and reproducibility were for Dumas 1.48 and 2.01% and Kjeldahl 1.62 and 2.37%, respectively. A single analysis conducted in 2 laboratories should not differ by more than 5.63% of the mean value when measured by Dumas or by more than 6.64% by Kjeldahl. It is concluded that with fishmeal, Dumas gives a more reliable measure of organic nitrogen than Kjeldahl, and, therefore, Dumas should be the method of choice.

The determination of nitrogen in fishmeal is critical for daily quality control of production and for specification in contracts. All fishmeal is traded on its protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum quantity of protein present.

The traditional protein determination by Kjeldahl is relatively accurate, but it is time-consuming; exposes the analyst to toxic fumes, concentrated acid, and alkali; and produces chemical wastes that must be disposed. The automated Kjelfoss method is somewhat quicker than the Kjeldahl method, but it still requires catalyst and other chemicals.

It has been well documented that the nitrogen determination by combustion method (Dumas) with determination of released N_2 by thermal conductivity is accurate and quick and obviates the need for hazardous and toxic chemicals. AOAC INTERNATIONAL (1) and the American Oil Chemists Society (AOCS; 2) have officially recommended generic standard methods for the determination of nitrogen in feeding stuffs as well as methods for specific feeds. The International Organization for Standardization (ISO) has a standard method for determination of nitrogen in milk and milk products (3) and has a method under publication for cereals, pulses, oilseeds, and animal feeding stuffs (4). However, no collaborative study of the Dumas method applied to fishmeals has been published; consequently, the International Fishmeal and Oil Manufacturers Association (IFOMA; subsequently continued by the International Fishmeal and Fish Oil Organization Ltd, IFFO) initiated this study as part of its review of standard methods recommended to its members.

Wiles et al. (5) reviewed 16 trials in which the Dumas and Kjeldahl methods were compared. This review indicated the Dumas method generally gave a slightly greater value, but there was no agreed relationship. These authors went on to

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Table 1. Moisture determinations (% sample) in each laboratory of hidden duplicate pairs of 4 meals and of 2 additional high-protein meals treated as a Youden pair

	Sample of fishmeal and country of origin									
	Peru		U.S. fish bone meal		Thailand		Alaska		Denmark	Peru
Lab	2	8	3	5	4	7	6	10	1	9
1	7.95	8.04	8.71	8.72	7.91	7.82	8.17	8.17	6.44	7.03
2	8.33	8.31	8.65	9.36	8.00	8.08	8.56	8.58	6.42	7.29
3	7.89	7.92	8.53	8.40	7.91	7.98	8.05	8.08	6.50	6.98
4	8.00	7.60	8.30	8.30	7.30	7.40	7.90	7.80	6.00	7.00
5	7.33	7.41	8.00	8.06	7.52	7.72	7.60	7.60	5.85	6.60
6	7.50	7.30	7.70	7.30	7.40	7.30	7.50	7.70	5.70	6.50
7	7.78	7.14	8.35[a]	9.49[a]	7.85[b]	6.63[b]	7.57	8.21	6.09	6.88
8	8.10	8.00	8.50	8.10	8.00	7.90	8.20	8.20	6.40	7.10
9	7.37	7.50	8.56	8.70	7.60	7.71	7.96	7.47	5.72	6.55
10	7.64	7.58	8.42	8.43	7.69	7.67	7.78	7.39	5.92	6.53
12	7.80	7.70	8.60	8.50	8.10	8.00	8.00	8.10	6.30	7.10
13	7.35	6.74	7.94	7.26	6.51[b]	6.42[b]	7.68	7.20	5.15	6.22
14	7.50	7.40	7.80	8.00	7.90	8.10	7.80	8.00	6.40	7.00
15	7.71	7.80	8.62	8.68	7.79	7.91	7.95	8.17	6.44	7.00
16	8.30	8.10	9.00	8.90	8.10	8.10	8.30	8.30	6.50	7.20
17	7.60	7.50	8.00	8.40	8.00	7.90	7.60	7.80	6.10	6.70
18	8.10	8.00	8.70	8.60	7.80	7.90	8.20	8.20	6.50	7.10
19	8.02	8.05	8.53	8.13	7.92[b]	8.94[b]	7.92	14.00[b]	6.21[b]	8.04[b]

[a] Values determined as stragglers ($0.01 < P < 0.05$).

[b] Outlier values ($P < 0.01$).

establish there was no difference in results between the 2 methods when applied to dairy products. In contrast, King-Brink and Sebranek (6) reported an interlaboratory study in which the Dumas method gave consistently greater values, though not significantly different, in 15 comparisons of meat and meat products. The mean bias over all participating laboratories and samples was 0.025% N, an increase of 1.03%. Similarly, Thompson et al. (7) observed higher values with Dumas with both canned meat (0.014% N, relative increase 0.7%) and canned fish (0.045% N, relative increase 2.0%). In an interlaboratory study of meat products, Thompson et al. (8) confirmed the bias was small but varied with product, which was significant for chicken where the bias was 0.02% N, a relative increase of 0.70%. A study with 20 Chilean fishmeals concluded that N measured by Kjelfoss in one laboratory correlated closely with results by Dumas using one particular machine in a second laboratory, the latter giving a result on average greater by 0.96% (9). The present study was planned as an interlaboratory study of the Dumas method as applied to fishmeals, including the use of a variety of equipment for the Dumas method. In addition, participating laboratories were asked to analyze the same samples by the Kjeldahl method to provide an interlaboratory comparison of means, repeatability, and reproducibility by the 2 methods. In anticipation that the Dumas method gives a greater result than Kjeldahl when applied to fishmeal, investigations were undertaken to establish possible reasons for the difference.

Interlaboratory Study

Organization of the Study

Six samples of fishmeal were used. These samples were commercially available on the world market and came from producers in Scandinavia, Peru, Thailand, and the United States (mainland and Alaska). They were selected to cover the whole range of crude protein likely to be met, from low-protein fish bone meal to high-protein meals. The meals were ground to pass through an ASTM No. 30-32 screen, which is 0.50–0.595 mm mesh. Ten 50 g samples of fishmeal were sent to 19 participating laboratories. The samples were coded 1 to 10, but the code for each laboratory differed. There were 4 hidden duplicates. The 2 meals not duplicated were the 2 highest protein meals, which were treated as a Youden pair (10) in the statistical analysis to determine repeatability, but as separate meals in the statistical analysis to determine reproducibility. Two 10 g samples of pure standards, tryptophan and nicotinic acid, were also sent as unknown

Table 2. Individual laboratory values determined by the Dumas method for N content (% sample) of hidden duplicate pairs of 4 meals, 2 additional high-protein meals (Nos. 1 and 9), and 2 unknown standards

	Sample of fishmeal and country of origin										Unknown standards	
	Peru		U.S. fish bone meal		Thailand		Alaska		Denmark	Peru	Tryptophan	Nicotinic acid
Lab	2	8	3	5	4	7	6	10	1	9	11	12
1	10.44	10.63	8.65	9.10	9.00	9.00	10.95	10.94	13.64	11.44	13.92	11.48
2	10.57	10.45	8.64	8.59	9.06	9.00	10.96	10.90	13.59	11.38	13.77	11.37
3	10.60	10.65	8.57	8.60	8.73	8.47	10.85	10.91	13.51	11.43	13.70	11.37
4	10.45	10.53	9.16	9.16	9.08	9.03	10.84	10.89	13.54	11.37	13.60	11.38
5	10.31	10.49	8.37	8.35	8.87	8.89	10.90	10.88	13.52	11.37	13.30	11.24
6	10.73[a]	10.27[a]	8.44	8.91	8.45	8.76	10.79	10.73	13.43	11.32	13.61	10.78
7	10.56	10.68	7.90	7.80	8.82	8.81	10.88	10.90	13.46	11.43	13.51	10.83
8	10.50	10.51	8.93	8.99	8.90	9.01	10.79	10.79	13.36	11.28	13.51	11.42
9	10.53	10.68	8.76	7.89	8.25[a]	8.78[a]	11.00	10.93	13.43	11.36	13.51	11.30
10	10.38	10.52	8.54	9.16	8.63	8.63	10.87	10.98	13.37	11.45	13.73	11.57
12	10.57	10.43	9.12	9.04	8.85	8.94	10.88	10.93	13.51	11.35	13.64	11.28
13	10.54	10.37	9.18	8.46	8.85	8.93	10.94	10.78	13.39	11.30	13.49	10.70
14	10.37	10.38	8.90	8.93	8.35	8.37	10.85	10.82	13.46	11.33	13.63	11.30
15	10.25	10.41	7.74	8.48	8.62	8.72	10.50[b]	10.72[b]	13.28	11.19	13.49	11.18
16	10.65	10.53	8.57	8.26	8.35	8.20	10.86	10.81	13.48	11.32	13.60	11.16
17	10.44	10.43	8.97	8.81	8.46	8.65	10.88	10.86	13.53	11.43	13.67	11.32
18	10.60	10.60	8.50	8.60	8.90	9.10	10.90	11.00	13.60	11.50	13.80	11.40
19	10.48	10.53	9.05	8.98	8.65	8.74	10.87[b]	10.15[b]	13.51	11.16	13.71	11.34

[a] Values determined as stragglers ($0.01 < P < 0.05$).

[b] Outlier values ($P < 0.01$).

Table 3. Individual laboratory values determined by the Dumas method, expressed as % of dry matter, for N content of hidden duplicate pairs of 4 meals and 2 additional high-protein meals (Nos. 1 and 9)

	Sample of fishmeal and country of origin									
	Peru		U.S. fish bone meal		Thailand		Alaska		Denmark	Peru
Lab	2	8	3	5	4	7	6	10	1	9
1	11.34	11.56	9.48	9.97	9.77	9.76	11.92	11.91	14.58	12.31
2	11.53	11.40	9.46	9.48	9.85	9.79	11.99	11.92	14.52	12.27
3	11.51	11.57	9.37	9.39	9.48	9.20	11.80	11.87	14.45	12.29
4	11.36	11.40	9.99	9.99	9.80	9.75	11.77	11.81	14.40	12.23
5	11.13	11.33	9.10	9.08	9.59	9.63	11.80	11.77	14.36	12.17
6	11.60[a]	11.08[a]	9.14	9.61	9.13	9.45	11.66	11.63	14.24	12.11
7	11.45	11.50	8.62	8.62	9.57	9.44	11.77	11.87	14.33	12.27
8	11.43	11.42	9.76	9.78	9.67	9.78	11.75	11.75	14.27	12.14
9	11.37	11.55	9.58	8.64	8.93[a]	9.51[a]	11.95	11.81	14.24	12.16
10	11.24	11.38	9.33	10.00	9.35	9.35	11.79	11.86	14.21	12.25
12	11.46	11.30	9.98	9.88	9.63	9.72	11.83	11.89	14.42	12.22
13	11.38	11.12	9.97	9.12	9.47	9.54	11.85	11.62	14.12	12.05
14	11.21	11.21	9.65	9.71	9.07	9.11	11.77	11.76	14.38	12.18
15	11.11	11.29	8.47	9.29	9.35	9.47	11.41	11.67	14.19	12.03
16	11.61	11.46	9.42	9.07	9.09	8.92	11.84	11.79	14.42	12.20
17	11.30	11.28	9.75	9.62	9.20	9.39	11.77	11.78	14.41	12.25
18	11.53	11.52	9.31	9.41	9.65	9.88	11.87	11.98	14.55	12.38
19	11.39	11.45	9.89	9.77	9.39	9.60	11.80	11.80	14.40	12.14

[a] Values determined as stragglers ($0.01 < P < 0.05$).

materials. These were coded as 11 and 12, but the code for individual laboratories varied. The participating laboratories represented major fishmeal producers, end users, and independent commercial analytical laboratories in the United States, South America, Scandinavia, Korea, and Ireland, which routinely perform N analyses on fishmeal. Eighteen laboratories returned results. All laboratories performed analysis by the Dumas method using their own equipment and their own calibrating standards. Laboratories were asked to analyze the samples by the Kjeldahl method according to ISO 5983:1997(E) (11). In the outcome, 14 laboratories reported results using a variety of methods, presumably those in routine use in their laboratories. Four laboratories did not do the analysis. Participating laboratories were asked to analyze moisture in all 10 samples of fishmeal according to ISO 6496:1983 (12). Additional information on the Dumas equipment used, variant of the Kjeldahl procedure and catalyst used, and general comments were requested on the report form.

Analytical Protocol

A detailed protocol for conducting the ring test was circulated to laboratories. This included detailed instructions for conduct of the Dumas method as well as general instructions for the determination of nitrogen by Kjeldahl and of moisture. It was known that some laboratories calibrate the Dumas equipment using a sample of known nitrogen content determined by the Kjeldahl method, thereby automatically adjusting the Dumas value to be equivalent to the Kjeldahl value. Participants were instructed not to calibrate the equipment in this way for the collaborative determination but to use a high-purity standard of known nitrogen content. Participants were instructed to conduct at least 3 blank determinations to zero the Dumas equipment and 10 standard determinations to check calibration and then to analyze all 12 samples once only and, finally, to analyze the standard 3 times to check for instrument drift.

Laboratories reported all results (g/100 g sample) to 2 decimal places and gave the name of the high-purity standard used to calibrate Dumas equipment.

Method for Determining Protein in Fishmeal by Dumas (Combustion)

Principle

The sample is burned under a flow of pure oxygen in a combustion tube according to Dumas. The interfering gases

Table 4. Individual laboratory values determined by the Kjeldahl method for N content (% sample) of hidden duplicate pairs of 4 fishmeals, 2 additional high-protein fishmeals, and 2 standards

	Sample of fishmeal and country of origin										Unknown standards	
	Peru		U.S. fish bone meal		Thailand		Alaska		Denmark	Peru	Tryptophan	Nicotinic acid
Lab	2	8	3	5	4	7	6	10	1	9	11	12
1	10.41	10.44	8.60	9.00	8.88	8.88	10.73	10.76	13.28	11.22	13.52	8.99
4	10.35	10.51	9.14	9.14	9.04	9.01	10.79	10.83	13.34	11.24	13.44	5.29
5	10.24	10.22	8.35	8.32	8.85	8.83	10.82	10.82	13.39	11.31		
7	10.17	10.32	7.77	7.54	8.20	8.65	10.53	10.78	13.06	10.97	13.17	8.39
8	10.37	10.37	8.83	8.82	8.74	8.84	10.66	10.63	13.23	11.06	13.38	11.17
9	10.32	10.42	8.66	7.75	8.14	8.61	10.76	10.64	13.17	11.11	13.38	11.14
10	10.12	10.31	7.93	8.65	8.52	8.41	10.65	10.41	12.20[a]	11.09	12.22[a]	2.99
12	10.50	10.24	9.06	9.06	8.80	8.99	10.80	10.64	13.12	11.28	13.65	5.38
13	10.45	10.08	8.99	8.46	8.70	8.90	10.67	10.61	13.15	11.06	13.47	10.64
14	10.45	10.43	8.82	8.96	8.38	8.43	10.90	10.74	13.49	11.31	13.65	11.28
15	10.21	10.34	7.78	8.46	8.78	8.70	10.56	10.77	13.24	11.17	12.71	
17	10.46	10.50	9.19	8.89	8.48	8.68	10.93	10.95	13.55	11.39	13.65	10.68
18	10.50	10.50	8.40	8.40	8.90	8.90	10.80	10.80	13.40	11.30	13.30	4.80
19	10.39	10.41	9.00	8.96	8.54	8.59	10.75[a]	9.72[a]	13.29	11.03	13.57	11.05

[a] Outlier values ($P < 0.01$).

Table 5. Individual laboratory values determined by the Kjeldahl method, expressed as % of dry matter, for N content of hidden duplicate pairs of 4 meals and 2 additional high-protein meals (Nos. 1 and 9)

	Sample of fishmeal and country of origin									
	Peru		U.S. fish bone meal		Thailand		Alaska		Denmark	Peru
Lab	2	8	3	5	4	7	6	10	1	9
1	11.31	11.35	9.42	9.86	9.64	9.63	11.68	11.72	14.19	12.07
4	11.25	11.37	9.97	9.97	9.75	9.73	11.72	11.75	14.19	12.09
5	11.05	11.04	9.08	9.05	9.57	9.57	11.71	11.71	14.22	12.11
7	11.03	11.11	8.48	8.33	8.90	9.26	11.39	11.74	13.91	11.78
8	11.28	11.27	9.65	9.60	9.50	9.60	11.61	11.58	14.13	11.91
9	11.14	11.26	9.47	8.49	8.81	9.33	11.69	11.50	13.97	11.89
10	10.96	11.16	8.66	9.45	9.23	9.11	11.55	11.24	12.97[a]	11.86
12	11.39	11.09	9.91	9.90	9.58	9.77	11.74	11.58	14.00	12.14
13	11.28	10.81	9.77	9.12	9.31	9.51	11.56	11.43	13.86	11.79
14	11.30	11.26	9.57	9.74	9.10	9.17	11.82	11.67	14.41	12.16
15	11.06	11.21	8.51	9.26	9.52	9.45	11.47	11.73	14.15	12.01
17	11.32	11.35	9.99	9.71	9.22	9.42	11.83	11.88	14.43	12.21
18	11.43	11.41	9.20	9.19	9.65	9.66	11.76	11.76	14.33	12.16
19	11.30	11.32	9.84	9.75	9.27	9.43	11.67	11.30	14.17	11.99

[a] Outlier values ($P < 0.01$).

are removed and nitrogen oxides are reduced to nitrogen, which is quantitatively determined using a thermal conductivity cell. Total nitrogen content is given by a microprocessor in a g/100 g sample after proper calibration of the instrument.

Apparatus and Supplies

(**a**) *Analytical balance*.—Capable of weighing to 0.10 mg. (*Note*: A balance may be provided by the instrument and might be incorporated into the microprocessor of the system.)

(**b**) *Grinding device.*—Capable of grinding the fishmeal to an ASTM No. 30-32 screen (0.50–0.595 mm).

(**c**) *Dumas combustion apparatus*.—With thermal conductivity detector and suitable device for signal integration. Use according to manufacturer's manual.

Nitrogen-Free Reagents and Gases

(*Note*: Most of the reagents are supplied by the instrument manufacturer under proprietary names.)

(**a**) *Oxygen.*—99.99% pure.

(**b**) *Helium.*—99.99% pure OR.

(**c**) *Carbon dioxide.*—99.99% pure.

(**d**) *Compressed air.*

(**e**) *Aluminium oxide*.—For the combustion tube.

(**f**) *Magnesium perchlorate or phosphorus pentoxide*.—For dehydrating agent.

(**g**) *Sodium hydroxide on silica.*—For carbon dioxide absorption.

(**h**) *Magnesium dioxide or silver tungstate.*—For the reduction tube.

(**i**) *Copper granules*.—To fill the reduction tube.

(**j**) *Copper turnings.*—For the reduction tube.

(**k**) *Tin foil or cellulose capsules or boats.*—For the introduction of the samples. (*Note*: Some laboratories use small samples and the ash generated by tin foil requires frequent cleaning of the system. Cellulose capsules obviate this problem.)

(**l**) *Glass wool.*

(**m**) *Sulfur dioxide absorbent.*—Such as lead chromate.

(**n**) *Copper oxide–platinum catalyst*.—As filling material of the postcombustion tube.

(**o**) *High-purity nitrogen standard*.—Purified reagent grade ethylenediaminetetraacetic acid (EDTA), 9.59% N; nicotinic acid, 11.38% N; or tryptophan, 13.72% N. Store in a desiccator.

Procedure

(**a**) Grind the sample to pass an ASTM No. 30-32 screen (0.500–0.595 mm). For the interlaboratory study, samples were preground prior to distribution.

(**b**) Following the manufacturer's directions, run the instrument to first purge any nitrogen from the system. This is a blank determination designed to zero the instrument response. The blank determination should be run whenever the instrument has been stopped over a period of 1 h. Run at least 3 analyses without sample. The blank value should be stable, and the instrument should be zeroed if necessary.

Table 6. Recovery (%) of hidden standards analyzed by the Dumas and Kjeldahl methods, together with reproducibility standard deviation (s_R) and reproducibility relative standard deviation (RSD_R) values

	Dumas		Kjeldahl	
Lab	Tryptophan	Nicotinic acid	Tryptophan	Nicotinic acid
1	101.5	100.9	98.6	79.0
2	100.4	99.9		
3	99.9	99.9		
4	99.1	100.0	98.0	46.5
5	96.9	98.8		
6	99.2	94.7		
7	98.5	95.2	96.0	73.7
8	98.5	100.4	97.5	98.2
9	98.5	99.3	97.5	97.9
10	100.1	101.7	89.1	26.3
12	99.4	99.1	99.5	47.3
13	98.3	94.0	98.2	93.5
14	99.4	99.3	99.5	99.1
15	98.3	98.3	92.6	
16	99.1	98.1		
17	99.6	99.5	99.5	93.9
18	100.6	100.2	96.9	42.2
19	99.9	99.7	98.9	97.1
Mean	99.3	98.8	97.1	74.6
s_R	1.04	2.11	3.03	26.76
RSD_R	1.05	2.14	3.13	35.90

(**c**) Following the manufacturer's directions and assuming that the instrument response is linear, check the calibration using the mean of 10 successive results from the analysis of one of the high-purity standards of known nitrogen content. The mean should be within 0.05% nitrogen (0.31% protein) of the reference value for that standard.

(**d**) According to the manufacturer's directions, weigh the appropriate amount of sample and place into the combustion chamber.

Note: Some of the more automated units allow the blanks, standards, and samples all to be incorporated into an automatic feeder. The instrument then goes through the blanks, standards, and samples. Prior to calculation of results, the analyst zeros the instrument, corrects for the standard, and then the computer calculates the results.

(**e**) Analyze test portions according to the manufacturer's directions.

(**f**) Record the results calculated as N g/100 g sample to 2 decimal places.

(**g**) Calculate crude protein g/100 g sample as N × 6.25.

Statistical Assessment of Results

Statistical procedures for the determination of repeatability and reproducibility were in accordance with ISO 5725-2:1994(E) (13). In addition, the 2 high-protein fishmeals, Nos. 1 and 9, and the 2 standards, Nos. 11 and 12, were treated as Youden pairs to provide estimates of repeatability standard deviations (s_r; 10) in addition to those obtained from the hidden duplicate samples. Reproducibility standard deviations (s_R) were calculated from the totals of the hidden duplicates and also from the individual Meals 1 and 9 and Standards 11 and 12. Data from each material (fishmeal or standards) were examined separately to identify outlier laboratories by Cochran's and Grubb's tests (13) using $P < 0.01$ to define an outlier and probability $0.05 > P > 0.01$ to define a straggler. Cochran's test of the homogeneity of within-laboratory variance determines whether the laboratory with the largest variance between duplicates accounts for a significant proportion of the total of the variances for all laboratories. Grubb's test determines whether the highest or lowest laboratory mean value differs significantly from the mean of all laboratories. Repeatability and reproducibility standard deviations were calculated for each material using both the full data and after exclusion of outlier values. Where appropriate, pooled estimates of s_r and s_R were calculated as the square root of the averaged variances weighted by the appropriate degrees of freedom, not as the simple average as stated in ISO 5725-2:1994(E) (13). Comparison of results by Dumas and Kjeldahl was determined from the difference within each sample and laboratory. The variance of these differences was determined across samples to provide a pooled estimate for each laboratory and across laboratories to provide a within-sample pooled estimate for each material and a pooled overall variance weighted by degrees of freedom. Data management, Cochran's and Grubb's tests, and computation of the various sources of variation for each material were performed in Excel (Microsoft Corp., Redmond, WA) and using the statistical package GenStat for Windows Release 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts, UK).

Results and Discussion

Initial Screening of Data for Outliers

Laboratory 2 reported that the Dumas equipment had been standardized against a feed sample and not an absolute standard as required in the protocol. This laboratory did not undertake a Kjeldahl analysis, and, therefore, its results would not contribute to a direct comparison of the 2 methods. However, the Dumas values were close to the mean value of the remaining laboratories. Exclusion of Laboratory 2 values consequently had very little effect on the means and only slightly increased the repeatability and reproducibility standard deviations. Consequently, the data were retained in the analysis of the moisture and Dumas values.

(**a**) *Moisture content*.—Table 1 gives the reported moisture values for each of the 10 fish samples. Results identified as outliers or stragglers are indicated. Laboratory 19

Table 7. Mean, repeatability standard deviation (s_r), and repeatability relative standard deviation (RSD_r) values of moisture determination (% sample) for each fishmeal material together with pooled estimates for all 6 fishmeals, including the Youden pair, before and after excluding outliers

Sample	Fishmeal Peru 2, 8	Fish bone meal U.S. 3, 5	Fishmeal Thailand 4, 7	Fishmeal Alaska 6, 10	Fishmeals 1 and 9 Youden pair	Pooled 6 fishmeals
			All data			
Mean	7.73	8.40	7.74	8.10	6.54	7.70
s_r	0.176	0.290	0.275	1.030	0.214	0.509
RSD_r	2.28	3.45	3.55	12.71	3.27	6.61
			Excluding outliers			
Mean	7.73	8.40	7.82	7.94	6.51	7.68
s_r	0.176	0.290	0.079	0.190	0.113	0.188
RSD_r	2.28	3.45	1.01	2.39	1.74	2.44

reported that it took a long time for the samples to get through customs, that it seemed some of the samples had been opened, there was water in the package, and Sample No. 10 was damaged. Indeed, the reported moisture content of Sample 10 was clearly much greater than its hidden duplicate, Sample 6, and the variance of these 2 samples was a clear outlier by the Cochran test ($P < 0.01$). Furthermore, while Sample 10 was a clear outlier by the Grubb's test, Sample 6 was not. Thus, for Laboratory 19, Sample 10 was treated as an outlier but Sample 6 was retained in the data set. The moisture values reported by Laboratory 19 for the hidden duplicate pair Samples 4 and 7 also had high variance, which was a significant outlier by the Cochran test ($P < 0.01$). This pair was also treated as an outlier. The moisture values reported by Laboratory 19 for the Youden pair of meals, Samples 1 and 9, also had high variance of the difference between meals, corrected for the mean difference over all laboratories, significant by Cochran's test ($P < 0.01$). As the variance could not be attributed solely to one of the meals, values for both Samples 1 and 9 were treated as outliers. Laboratory 7 reported values for the hidden duplicate pair Samples 4 and 7, which had high variance, significant by Cochran's test ($P < 0.01$). This variance could not be attributed to either sample alone, so the pair was treated as outliers. Laboratory 7 also reported a high variance for the hidden pair Samples 3 and 5 (Cochran test $0.05 > P > 0.01$). As this was not attributable to any one sample, both results were retained as stragglers. Laboratory 13 reported low values for the hidden pair Samples 4 and 7. The mean was identified as an outlier by Grubb's test ($P < 0.01$), with Sample 4 also an outlier ($P < 0.01$) and Sample 7 a straggler ($0.05 > P > 0.01$) when tested on each sample separately. Both values were treated as outliers.

(**b**) *Nitrogen by Dumas*.—Tables 2 and 3 give the reported values on a fresh weight basis and, after correction for the corresponding moisture content (Table 1), a dry matter basis, respectively. Values identified as stragglers and outliers are indicated. On a fresh weight basis, for the hidden pair, Samples 6 and 10, Laboratory 19 was an outlier due to high variance (Cochran test). When this pair was eliminated, the average value for the pair returned by Laboratory 15 was an outlier due to a low value (Grubb's test). However, when expressed on a dry matter basis, both of these paired values were within the normal range. Thus, there were no outliers.

In contrast, Laboratory 6 returned values for the hidden pair, Samples 2 and 8, that were stragglers on both a fresh weight and dry matter basis by the Cochran test. Similarly, Laboratory 9 returned values for the hidden pair, Samples 4 and 7, that were stragglers on both a fresh weight and dry matter basis by the Cochran test. Results identified as stragglers were not excluded.

(**c**) *Nitrogen by Kjeldahl*.—Tables 4 and 5 give the reported values on a fresh weight basis and a dry matter basis, respectively. Values identified as stragglers and outliers are indicated. Again, Laboratory 19 values for Samples 6 and 10 were outliers by the Cochran test on a fresh weight basis but not when corrected to a dry matter basis. Laboratory 10 returned a low value for Sample 1. This led to a significantly increased variance by the Cochran test when considered as a Youden pair with Sample 9 and was significantly different from the other results for Sample 1 by Grubb's test. The same result was obtained on a dry matter basis. Laboratory 10 returned the lowest values for tryptophan (Sample 11), which was a straggler by the Grubb's test but was close to being classed as an outlier (Grubb's statistic 2.634 versus a critical value of 2.699). When considering both standards as a Youden pair, Laboratory 15 is unpaired and, therefore, was omitted. In this situation, Laboratory 10 then becomes a very significant outlier for tryptophan. Consequently, Laboratory 10 was finally classified as an outlier for tryptophan. Table 4 also indicates that most laboratories had major difficulties in determining the correct nitrogen content

Table 8. Mean, reproducibility standard deviation (s_R), and reproducibility relative standard deviation (RSD_R) values of moisture determination for each material (% sample) together with pooled estimates for the 4 hidden duplicates and all 6 fishmeals before and after excluding outliers

Sample	Fishmeal Peru 2, 8	Fish bone meal U.S. 3, 5	Fishmeal Thailand 4, 7	Fishmeal Alaska 6, 10	Fishmeal Denmark 1	Fishmeal Peru 9	Pooled 6 fishmeals
All data							
Mean	7.73	8.40	7.74	8.10	6.15	6.93	7.51
s_R	0.362	0.476	0.478	1.061	0.369	0.399	0.602
RSD_R	4.68	5.67	6.17	13.10	6.01	5.75	8.01
Excluding outliers							
Mean	7.73	8.40	7.82	7.94	6.14	6.87	7.48
s_R	0.362	0.476	0.245	0.333	0.380	0.297	0.362
RSD_R	4.68	5.67	3.13	4.19	6.19	4.32	4.84

of the standard nicotinic acid (Sample 12). Laboratory 15 did not return a value. For nicotinic acid, determined values ranged from 11.28 to 2.99% compared with a theoretical value of 11.38%. Laboratory 10 returned the lowest value. The variability was so great that no values were identified as outliers, either in the Cochran test of variance as part of a Youden pair with tryptophan, or as a Grubb's test on Sample 12 alone. Because the 2 standards clearly differed in their ease of digestion in the Kjeldahl method, the use of the Youden pair method to estimate repeatability is inappropriate.

In summary, Laboratory 19 (Samples 6 and 10) and Laboratory 10 (Samples 1 and 11) were identified as outliers when determined on a fresh weight basis. On a dry weight basis, only Laboratory 10 (Samples 1 and 11) remained as an outlier.

Analysis of Standards

Table 6 gives the recovery of nitrogen from tryptophan and nicotinic acid in the Dumas and, where reported, Kjeldahl methods. For the Dumas method, the overall recovery for tryptophan was 99.3%, with only Laboratory 5 returning a low value, 96.9%. For nicotinic acid, the overall recovery was 98.8%, but 3 laboratories (Nos. 6, 7, and 13) returned values of 95% or less. By Kjeldahl, the mean recovery of tryptophan was 97.1%, but Laboratory 10 returned a value of 89.1% and was classified as an outlier. Excluding Laboratory 10, the mean nitrogen recovery from tryptophan was 97.7%. Only 4 out of 12 laboratories returned a satisfactory value (>97%) for nicotinic acid. The conditions used by the best and worst 4 laboratories were scrutinized. Clearly, a range of different Kjeldahl methods were used and not solely ISO 5983:1997(E) (11) specified in the protocol. However, similar methods (e.g., ISO 5983) and catalysts (e.g., K_2SO_4, HgO, $CuSO_4$, and TiO_2) occurred within both subgroups of laboratories. Consequently, it is not possible to account for the different results in terms of method or catalyst. Similar difficulties in the Kjeldahl analysis of nicotinic acid have been reported previously (5). The temperature and time of digestion are important factors when catalysts other than mercury are used (Miller, E.L., unpublished information). According to Wiles et al. (5), if a laboratory cannot achieve a recovery of a standard of tryptophan or lysine HCl of 98% in the Kjeldahl method, its results for the test samples should be excluded in comparison with the Dumas method, otherwise a bias in favor of the Dumas method is created. On this basis, Laboratory 10 should be excluded from the entire data set. Indeed, Laboratory 10 generally provided low results by Kjeldahl for the fishmeal samples, being the lowest laboratory for the paired Samples 6 and 10 (but not an outlier or straggler) and for Sample 1 (an outlier). However, the values returned by this laboratory for the Dumas method were in the center of the range, and it would be inappropriate to delete these data from the assessment of the variability of the Dumas method per se. However, for the within-laboratory comparison of Dumas versus Kjeldahl, the analysis has been performed both with and without inclusion of Laboratory 10 where it was not already excluded as an outlier. If the same criterion were applied to the Dumas method, then Laboratory 5 should be excluded from the data. However, this laboratory analyzed the more difficult nicotinic acid within the 98% recovery limit by the Dumas method. As this laboratory did not report results by Kjeldahl, it is automatically not a component of the within-laboratory comparison of the methods.

Repeatability and Reproducibility of Moisture Determination

Tables 7 and 8 give the means and estimates of repeatability and reproducibility for moisture determination using all the data and excluding outlier values. Removing the outliers greatly reduced the variability, but the remaining data contained substantial variability. The pooled estimates over all samples of relative standard deviation of repeatability (RSD_r) and relative standard deviation of reproducibility (RSD_R) were 2.44 and 4.84, respectively. Thus, the same sample

Table 9. Mean, repeatability standard deviation (s_r), and repeatability relative standard deviation (RSD_r) values of N determination by the Dumas method for each material together with pooled estimates for the 6 fishmeals before and after excluding outliers

Sample	Fishmeal Peru 2, 8	Fish bone meal U.S. 3, 5	Fishmeal Thailand 4, 7	Fishmeal Alaska 6, 10	Fishmeals 1 and 9 Youden pair	Pooled 6 fishmeals	Standards 11, 12 Youden pair
All data, g N/100 g fresh weight							
Mean	10.50	8.67	8.75	10.85	12.42	10.24	12.43
s_r	0.112	0.278	0.129	0.133	0.062	0.160	0.150
RSD_r	1.07	3.21	1.47	1.23	0.50	1.56	1.21
Excluding outliers, g N/100 g fresh weight							
Mean	10.50	8.67	8.75	10.88	12.42	10.24	12.43
s_r	0.112	0.278	0.129	0.048	0.062	0.152	0.150
RSD_r	1.07	3.21	1.47	0.44	0.50	1.48	1.21
All data, g N/100 g dry matter							
Mean	11.38	9.46	9.48	11.80	13.28	11.08	
s_r	0.128	0.306	0.144	0.073	0.056	0.167	
RSD_r	1.13	3.23	1.52	0.62	0.42	1.51	

analyzed once in 2 different laboratories could differ by up to 13.55% (2.8 × 4.84) of the mean value in 95% of analyses. This variation may reflect either (*1*) real differences due to change of moisture content when the samples are shipped, (*2*) differences in analytical accuracy, or (*3*) both. If (*1*), the concentration of other analytes such as N expressed on a fresh weight basis will be directly affected, and the variability of such analyses cannot be expected to be better than that of the moisture variation. If (*1*), correction of other analyses to a dry matter basis will remove this source of variation. If (*2*), correction of other analyses to a dry matter basis will increase the variability by adding the variance of the moisture analysis to that of the analyte analysis.

Consequently, when samples are moved from one laboratory to another, and particularly between countries with large differences in humidity, changes in moisture content of samples can be expected. This can contribute substantially to the variability of the results for other analyzed components, such as nitrogen. Care should be taken to both minimize any changes in moisture content, to analyze for moisture content at the same time as other analyses are performed, and to conduct the moisture analysis according to defined procedures with high repeatability and reproducibility.

Repeatability and Reproducibility of Nitrogen Determination by the Dumas Method

Tables 9 and 10 give the means and estimates of repeatability and reproducibility for nitrogen determined by Dumas, using both all the data and excluding outlier values. Correction of the data to a dry matter basis obviated the need to remove outliers, although 2 other pairs of stragglers in the fresh weight data remained stragglers in the dry matter data. As seen in Tables 9 and 10, repeatability and reproducibility were more variable for the lower protein meals. Negative relationships between variability and N content were best described by exponential equations. However, fish bone meal (U.S.) was considerably more variable than the other materials. When this meal was excluded, apparent linear negative relationships with N content, accounting for 81 and 53% of the variation in s_r and s_R, respectively, remained, but the relationships failed to reach significance (P = 0.10 and 0.17, respectively). The higher variability with low-protein, high-ash meals may reflect sampling difficulties with more heterogeneous material. In this study, this should have been minimized by grinding through a 0.5 mm screen. Pregrinding the sample before distribution should have minimized any contribution to the between-laboratory variance from this source. The estimate of repeatability obtained with the Youden pair of high-protein meals was numerically less, but of a similar order of magnitude, compared to the estimates made from hidden duplicates. However, a direct comparison is confounded by the greater content of N of this pair and, therefore, an expected lower variability. Despite the demonstrated effect of N level, pooled estimates were calculated combining the 6 fishmeals to indicate general values that could be applied to all types of fishmeal. On a fresh weight basis, after excluding outliers, the best estimate of RSD_r is 1.48%. On a dry matter basis, without the need to exclude outliers, the corresponding value is 1.51%. The corresponding best estimates of RSD_R were 2.01 and 2.06% on fresh weight and dry matter, respectively. The additional moisture determination made a substantial correction in

Table 10. Mean, reproducibility standard deviation (s_R), and reproducibility relative standard deviation (RSD_R) values of N determination by the Dumas method for each material together with pooled estimates for the 6 fishmeals before and after excluding outliers

Sample	Fishmeal Peru 2, 8	Fish bone meal U.S. 3, 5	Fishmeal Thailand 4, 7	Fishmeal Alaska 6, 10	Fishmeal Denmark 1	Fishmeal Peru 9	Pooled 6 fishmeals	Standard tryptophan 11	Standard nicotinic acid 12
All data, g N/100 g fresh weight									
Mean	10.50	8.67	8.75	10.85	13.48	11.36	10.60	13.62	11.25
s_R	0.116	0.403	0.252	0.151	0.092	0.088	0.216	0.143	0.241
RSD_R	1.11	4.65	2.88	1.39	0.68	0.78	2.03	1.05	2.14
Excluding outliers, g N/100 g fresh weight									
Mean	10.50	8.67	8.75	10.88	13.48	11.36	10.61	13.62	11.25
s_R	0.116	0.403	0.252	0.116	0.092	0.088	0.213	0.143	0.241
RSD_R	1.11	4.65	2.88	1.07	0.68	0.78	2.01	1.05	2.14
All data, g N/100 g dry matter									
Mean	11.38	9.46	9.48	11.80	14.36	12.20	11.45		
s_R	0.145	0.429	0.268	0.112	0.127	0.090	0.229		
RSD_R	1.28	4.53	2.83	0.95	0.88	0.74	2.06		

certain cases, while the additional error of a second analysis only slightly increased the repeatability estimate. Thus, the same sample analyzed once for N (and moisture) in 2 laboratories should not differ by more than 5.63% (2.8 × 2.01) of the mean value on a fresh weight basis, and 5.77% (2.8 × 2.06) when expressed on a dry matter basis, in 95% of analyses. In absolute terms (reproducibility limit), a single crude protein determination made in 2 laboratories and expressed on a fresh weight basis should not differ by more than 3.73% units of crude protein in 95% of cases (2.8 × 0.213 × 6.25). When expressed on a dry matter basis, the maximum difference is 4.00% units of protein (2.8 × 0.229 × 6.25).

These values are comparable with published values of relative repeatability and reproducibility standard deviations (RSD_r range 0.77–3.50 and RSD_R range 1.24–3.66) for cereal grains, oilseeds, and meat and meat products (6, 14). Sweeney (15) reported lower values of RSD_r (0.59) and RSD_R (1.10) averaged over 14 animal diets and feeds (including meat and bone meal), but these values resulted after deleting 2 out of 9 laboratories as outliers, primarily for deviations in the accompanying Kjeldahl analysis. Furthermore, the RSD_R values reported by Sweeney (15), which are also the basis for and are reported in AOAC Method **990.03** (1), were wrongly calculated. They are too high because they were calculated as:

$$\sqrt{(s_r^2 + 2s_L^2)}/\text{mean} \times 100 \text{ instead of } \sqrt{(s_r^2 + s_L^2)}/\text{mean} \times 100$$

where s_L^2 is the variance component due to laboratory, including the laboratory by sample interaction. The values for meat and bone meal reported by Sweeney (15) are of particular interest as being a material of close similarity to fishmeal. A reanalysis of the values for meat and bone meal, including all 9 laboratories, gives RSD_r 0.49 and RSD_R 0.94, both substantially less than the current estimates for fishmeals of similar protein content. This may reflect the fewer number of laboratories that used analyzers from one manufacturer and predominately the same model compared with 15 laboratories using 5 different analyzer models from one manufacturer and 3 laboratories using an analyzer from a different manufacturer. Collaborative assays of the Dumas method applied to animal feedingstuffs have been undertaken by ISO, but the results have not yet been published (4).

Repeatability and Reproducibility of Nitrogen Determination by the Kjeldahl Method

Tables 11 and 12 give the means and estimates of repeatability and reproducibility for nitrogen determined by Kjeldahl both using all the data and excluding outlier values. Correction of the data to a dry matter basis reduced the number of outliers. Again fish bone meal (U.S.) produced greater variability. The relationships of variability with N content were similar to, but less significant than, those for Dumas. On a fresh weight basis, after excluding outliers, the best RSD_r is 1.62%. On a dry matter basis, the corresponding value is 1.66%. The corresponding best estimates of RSD_R were 2.37 and 2.39% on fresh weight and dry matter, respectively. Thus, the same sample analyzed once for N (and moisture) in 2 laboratories should not differ by more than 6.64% (2.8 × 2.37) of the mean value expressed on a fresh weight, and 6.69% (2.8 × 2.39) of the mean value expressed on a dry matter basis, in 95% of analyses. In absolute terms

Table 11. Mean, repeatability standard deviation (s_r), and repeatability relative standard deviation (RSD_r) values of N determination by the Kjeldahl method for each material together with pooled estimates for the 6 fishmeals before and after excluding outliers

Sample	Fishmeal Peru 2, 8	Fish bone meal U.S. 3, 5	Fishmeal Thailand 4, 7	Fishmeal Alaska 6, 10	Fishmeals 1 and 9 Youden pair	Pooled 6 fishmeals	Standards 11, 12 Youden pair
All data, g N/100 g fresh weight							
Mean	10.36	8.60	8.69	10.69	12.19	10.11	10.93[a]
s_r	0.107	0.294	0.143	0.215	0.198	0.202	2.002[a]
RSD_r	1.03	3.41	1.65	2.01	1.62	2.00	18.32[a]
Excluding outliers, g N/100 g fresh weight							
Mean	10.36	8.60	8.69	10.73	12.24	10.12	11.23[a]
s_r	0.107	0.294	0.143	0.096	0.069	0.164	1.838[a]
RSD_r	1.03	3.41	1.65	0.89	0.56	1.62	16.37[a]
All data, g N/100 g dry matter							
Mean	11.22	9.39	9.42	11.64	13.04	10.94	
s_r	0.122	0.322	0.145	0.137	0.205	0.200	
RSD_r	1.08	3.43	1.54	1.18	1.57	1.83	
Excluding outliers, g N/100 g dry matter							
Mean	11.22	9.39	9.42	11.64	13.09	10.95	
s_r	0.122	0.322	0.145	0.137	0.070	0.182	
RSD_r	1.08	3.43	1.54	1.18	0.53	1.66	

[a] Because the 2 standards behave differently in this analysis, it is not appropriate to classify them as a Youden pair. The values are given for illustration purposes only.

(reproducibility limit), a single crude protein determination made in 2 laboratories and expressed on a fresh weight basis should not differ by more than 4.34% units of crude protein in 95% of cases (2.8 × 0.248 × 6.25). When expressed on a dry matter basis, the maximum difference is 4.73% units of protein (2.8 × 0.270 × 6.25).

Comparison of Dumas and Kjeldahl Methods

Table 13 displays for each of the 6 fishmeals and the 2 standards the difference between the 2 methods (Dumas minus Kjeldahl) averaged over all of the relevant laboratories. In this analysis, data on a fresh weight basis were used, because differences between laboratories in moisture content do not affect the within-laboratory comparison of the 2 methods. In Table 13, part A, outlying data from Laboratory 10 for Sample 1 and tryptophan were removed. For each meal, Dumas gave a higher result ($P < 0.01$ or <0.001). The difference increased linearly with nitrogen content averaged over both methods ($P < 0.001$). The simplest relationship describing this difference is:

$$\text{Kjeldahl N, g/100 g meal} = 0.987 \text{ (s.e. } 0.0007) \times \text{Dumas N } (P < 0.001)$$

However, the average difference, especially as a percentage of the N value, is a useful descriptor (Table 13, part A). The pooled difference over all fishmeals, shown in Table 13, part A, was 0.128 g N/100 g meal (s.e. 0.0123; $P < 0.001$) or 1.26% (s.e. 0.122) of N. A similar difference (0.216 ± 0.0705; $P < 0.01$; 1.60 ± 0.521% of N) was observed with tryptophan. Because most laboratories failed to determine nicotinic acid correctly by Kjeldahl, the difference was very large (2.79 ± 0.905; $P < 0.01$; 28.3 ± 9.16% of N).

The analysis of the Dumas-Kjeldahl differences was repeated after omitting all values for Laboratory 10. The new estimates of mean difference are shown in Table 13, part B. These values are conservatively the best estimate of the difference between methods after exclusion of outliers and the questionable values from Laboratory 10. The difference increased linearly with nitrogen content averaged over both methods (Figure 1; $P < 0.001$). Using the 10 samples as independent estimates, the linear regression equation relating Kjeldahl to Dumas was:

$$\text{Kjeldahl N, g/100 g meal} = 0.973 \text{ (s.e. } 0.0041; P < 0.001) \times \text{Dumas N} + 0.165 \text{ (s.e. } 0.0428; P = 0.005)$$

Table 12. Mean, reproducibility standard deviation (s_R), and reproducibility relative standard deviation (RSD_R) values of N determination by the Kjeldahl method for each material together with pooled estimates for the 6 fishmeals before and after excluding outliers

Sample	Fishmeal Peru 2, 8	Fish bone meal U.S. 3, 5	Fishmeal Thailand 4, 7	Fishmeal Alaska 6, 10	Fishmeal Denmark 1	Fishmeal Peru 9	Pooled 6 fishmeals	Standard tryptophan 11	Standard nicotinic acid 12
All data, g N/100 g fresh weight									
Mean	10.36	8.60	8.69	10.69	13.21	11.18	10.46	13.32	8.48
s_R	0.122	0.487	0.239	0.226	0.322	0.129	0.283	0.416	3.045
RSD_R	1.18	5.65	2.75	2.12	2.44	1.15	2.71	3.13	35.90
Excluding outliers, g N/100 g fresh weight									
Mean	10.36	8.60	8.69	10.73	13.29	11.18	10.48	13.41	8.98
s_R	0.122	0.487	0.239	0.127	0.145	0.129	0.248	0.266	2.628
RSD_R	1.18	5.65	2.75	1.18	1.09	1.15	2.37	1.98	29.26
All data, g N/100 g dry matter									
Mean	11.22	9.39	9.42	11.64	14.07	12.01	11.29		
s_R	0.153	0.518	0.255	0.159	0.360	0.144	0.298		
RSD_R	1.36	5.51	2.71	1.36	2.56	1.20	2.64		
Excluding outliers, g N/100 g dry matter									
Mean	11.22	9.39	9.42	11.64	14.15	12.01	11.30		
s_R	0.153	0.518	0.255	0.159	0.179	0.144	0.270		
RSD_R	1.36	5.51	2.71	1.36	1.27	1.20	2.39		

This equation accounted for 100.0% of the variance in Kjeldahl values. Omitting the constant term enables the use of a simpler conversion factor: Kjeldahl N (or CP) = 0.989 (s.e. 0.0009) × Dumas N (or CP), $P < 0.001$, with very little loss of precision, because this still accounted for 100.0% of the variance. A similar proportionate difference of 0.984 × Dumas N was observed with tryptophan.

A comparative study within one laboratory of the Dumas and Kjeldahl methods applied to soybean products ranging from 1.5 to 90% protein also found the Dumas method to give higher values, the difference increasing with N content as indicated by the published regression equation; Kjeldahl CP = 0.971 × Dumas CP – 0.0175 (16). A reanalysis of the published data gives the absolute difference Dumas-Kjeldahl (g N/100 g meal) = 0.0293 (s.e. 0.00418) × mean N – 0.0091 (s.e. 0.0317), adjusted $r^2 = 0.873$, $P < 0.001$. The relationship between Kjeldahl and Dumas can be best given as Kjeldahl N (or CP) = 0.972 (s.e. 0.0024) × Dumas N (or CP; $P < 0.001$), indicating a greater proportionate difference between the methods than found in the current study. Jung et al. (16) produced the regression equation given above for the purpose of correcting crude protein by Dumas to the corresponding Kjeldahl value. However, this presumes the Kjeldahl value to be correct. Furthermore, the different equations found with soya products and fishmeals are as likely to reflect different laboratories and equipment involved in the comparison as difference in sample type. Consequently, the equation proposed by Jung et al. (16) cannot be regarded as an absolute correction factor that can be applied by all laboratories, even for soybean products.

As indicated above and in Tables 9–12, repeatability and reproducibility were generally better for the Dumas method. The F ratio of Kjeldahl variance/Dumas variance before exclusion of outliers and averaged over all fishmeals indicated better repeatability ($P = 0.019$) and reproducibility ($P = 0.005$) by Dumas. After exclusion of outliers, repeatability variance by Dumas was significantly better for sample pair Nos. 6 and 10 ($P = 0.005$), while averaged over all fishmeals the difference was not significant ($P = 0.240$). Similarly, after exclusion of outliers, reproducibility variance was significantly better for Meal 1 ($P = 0.040$), while averaged over all fishmeals the difference tended to be better for Dumas ($P = 0.079$).

Dumas as the Preferred Method

Because Dumas measures all nitrogen, in particular nitrate and nitrite, whereas Kjeldahl only measures organic nitrogen, attempts were made to measure the nitrate and nitrite in

Table 13. Comparison of N determination by the Dumas and Kjeldahl methods for 6 fishmeals and 2 standards before and after exclusion of Laboratory 10

Meal	Mean Dumas + Kjeldahl, g N/100 g meal	Difference Dumas – Kjeldahl, g N/100 g meal	Standard error of difference, g N/100 g meal	Probability	Percentage difference
All relevant laboratories (A)					
2, 8	10.422	0.128	0.0232	<0.001	1.23
3, 5	8.651	0.092	0.0314	<0.01	1.07
4, 7	8.736	0.088	0.0267	<0.01	1.01
6, 10	10.769	0.149	0.0291	<0.001	1.38
1	13.382	0.194	0.0395	<0.001	1.45
9	11.268	0.173	0.0351	<0.001	1.53
Mean	10.158	0.128	0.0123	<0.001	1.26
Tryptophan	13.515	0.216	0.0705	<0.01	1.60
Nicotinic acid	9.880	2.793	0.9053	<0.01	28.27
Excluding Laboratory 10 (B)					
2, 8	10.429	0.120	0.0241	<0.001	1.15
3, 5	8.657	0.057	0.0196	<0.01	0.65
4, 7	8.750	0.082	0.0283	<0.01	0.94
6, 10	10.772	0.130	0.0265	<0.001	1.21
1	13.382	0.194	0.0395	<0.001	1.45
9	11.268	0.158	0.0346	<0.001	1.41
Mean	10.187	0.113	0.0115	<0.001	1.11
Tryptophan	13.515	0.216	0.0705	<0.01	1.60
Nicotinic acid	10.116	2.267	0.7727	<0.05	22.41

fishmeal. Levels of nitrate plus nitrite would have to exceed 150 mg/kg (approximately 0.004 g N/100 g meal) before there is a noticeable difference (5). Duplicate samples of fishmeal No. 9 were sent to 3 laboratories. Only one was able to return results for which the duplicates were satisfactory. The mean values were 3 mg/kg nitrate-N and <0.3 mg/kg nitrite-N. Such a level, less than 0.1 of the discernible amount, cannot account for the difference observed in this interlaboratory study. Furthermore, under European legislation the nitrite content is limited to a maximum of 60 mg/kg as sodium nitrite, so this is unlikely to contribute to an elevated value by Dumas. Another possible source of bias is the inclusion of atmospheric nitrogen within bulky samples such as forages. This should not be a problem with dense fishmeal. The failure of a number of laboratories to recover N by Kjeldahl from nicotinic acid, and even from tryptophan, indicates the difference is due to incomplete digestion of organic N in the Kjeldahl method as currently used by the majority of laboratories. Lysine has also been shown to be more difficult to digest than tryptophan, with recovery values in the range of 87.1–91.8% compared with tryptophan at 97.4–99.2% under the same conditions (5). Because fishmeal is a rich source of lysine, it is possible that this will contribute to the observed difference between Dumas and Kjeldahl. If so, the use of Kjeldahl nitrogen will underestimate a most important contributor to the protein content of the meal. The Dumas value should be accepted as the correct value.

Conclusions

This study has shown, as have previous studies with other feedstuffs, that the Dumas method gives values of N for fishmeals that are 1.1% greater and have better repeatability and reproducibility than the Kjeldahl method. The same sample analyzed once for both N and moisture in 2 different laboratories should not differ by more than 5.77% (Dumas) or 6.69% (Kjeldahl) of the mean value expressed on a dry matter basis in 95% of analyses. The differences are unlikely to be attributable to contributions from inorganic N, such as nitrate and nitrite, but are related to incomplete digestion by Kjeldahl as shown by recoveries of less than 98% for tryptophan and extremely poor recoveries of nicotinic acid. In countries where the use of mercury as a catalyst in the Kjeldahl method is no longer permitted, alternative catalysts have to be substituted, but these also have disposal problems and are not as effective. In the present study, a variety of catalysts were used, including mercury, and this may contribute to the variability of the Kjeldahl results. It is recommended that laboratories using the Kjeldahl method should use standards of tryptophan and lysine

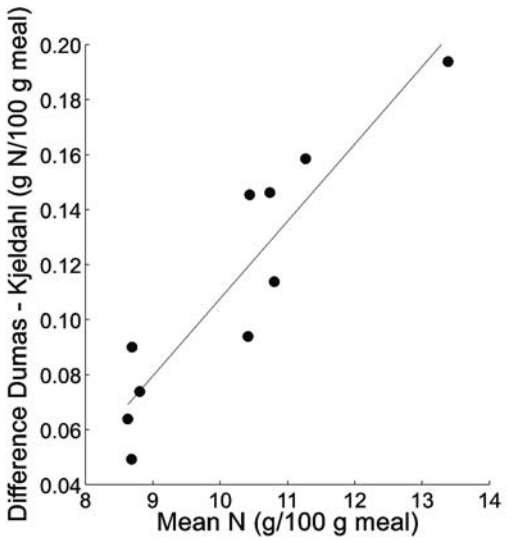

Figure 1. Relationship of the difference in N determination by Dumas or Kjeldahl and mean N content of fishmeals (g/100 g meal). The linear regression is shown: Dumas-Kjeldahl = 0.0281 (s.e. 0.00425) × N – 0.1736 (s.e. 0.0438), corrected r^2 = 0.826, *P* < 0.001.

HCl to check the recovery of N and adjust the use of catalyst, digestion temperature, and time to achieve at least 98% recovery. Now that an alternative, rapid, and nonpolluting Dumas method is available, the use of the Kjeldahl method should be phased out as soon as possible. IFFO has recommended that its members adopt the Dumas method as an official method for nitrogen and crude protein determination.

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