AOAC Official Method 968.06
Protein (Crude) in Animal Feed

Dumas Method

First Action 1968
Final Action 1969

A. Principle

N_2, freed by pyrolysis and subsequent combustions, is swept by CO_2 carrier into nitrometer. CO_2 is absorbed in KOH and volume residual N_2 is measured and converted to equivalent protein by numerical factor.

B. Apparatus and Reagents

(**a**) *Nitrogen analyzer and accessories*.—Consists of combustion and collection and measuring systems. Suitable instrument, Model 29A, with following accessories and reagents is available from Oak Brook Instruments Div. of Perkin Elmer Corp., 2000 York Rd, Oak Brook, IL 60521 (Perkin Elmer's current model of nitrogen analyzer is PE2410N nitrogen analyzer): Al combustion boats, No. 29–412; Vycor combustion tubes, No. 29–328; CuO-Pt catalyst (CuO wire form with 2.5% Pt reforming catalyst), No. 29–160; reduced Cu wire, No. 29–120; Co_3O_4, No. 29–170; CuO powder, fines, No. 29–140; 45% KOH, No. 29–110.

(**b**) *Balance*.—Accurate to 0.01 mg.

(**c**) *Barometer*.—Hg type, readable to 0.1 mm.

C. Preparation of Samples

Grind to pass No. 30 sieve. Store in capped bottles.

D. Determination

Operate instrument in accordance with instructions of manufacturer. (Following directions apply to Coleman Model 29A Nitrogen Analyzer. Consult Operating Directions D-360B, Coleman Cat. No. 29–904, for additional details.)

After combustion furnaces have come to thermal equilibrium, turn combustion cycle control to START and let proceed normally through cycle. Observe indicated temperature on pyrometer of both upper and lower combustion furnaces at end of combustion portion of cycle. Furnace temperatures should be 850–900°. If not, adjust.

Prepare combustion tube by inserting stainless steel screen in lower end of combustion tube (end farthest from trademark). In upper end, place enough glass wool to form 6 mm plug when packed. With 11 mm glass rod, drive glass wool down to stainless steel plug. Holding tube vertically, pour CuO-Pt catalyst directly from dispenser bottle into combustion tube until it reaches upper end of trademark. Tap or vibrate tube on bench until reagent settles to approximate center of trademark.

Weigh and record weight of empty Al combustion boat. Place sample in boat. Weigh and record weight of sample and combustion boat. Difference between weights is sample weight. Use following sample weights (mg) as guides to suitable sample sizes: bermuda grass 150–300; rice bran, wheat shorts, dehydrated alfalfa 150–250; range feed 100–200; cottonseed meal 75–150; edible soy protein 50–150. Weigh sample to nearest 0.01 mg. To avoid weight changes, record weight within 1 min after sample and boat are placed on balance. If this is impossible, weigh sample inside weighing bottle, such as Kimble No. 15165 or 15166.

Turn combustion tube to horizontal, and carefully insert loaded sample boat into open end of tube. Slide or push boat, without spilling contents, until it reaches trademark. Raise open end until tube forms 60–70° angle to horizontal. Tap or vibrate combustion tube on bench top while rotating tube between thumb and forefinger. Raise open end of tube and add volume Co_3O_4 and volume CuO fines equal to volume sample. For convenient means of adding above reagents to samples, place volume CuO fines and volume Co_3O_4, each equal to volume sample, in additional combustion boat; add contents of boat, but not boat itself, to combustion tube; and rotate partially filled combustion tube between thumb and forefinger while varying angle of tube 20–45° from horizontal. Continue rotating, tapping, and vibrating until sample is dispelled from boat and is thoroughly mixed with oxidizing agents. Raise open end until tube forms 60–70° angle to horizontal; add CuO-Pt catalyst ca 12 mm above sample boat. Tap or vibrate gently to eliminate voids. Add CuO-Pt catalyst to within 20 mm of top of tube, again tapping or vibrating gently to eliminate voids.

Install prepared combustion tube in N_2 analyzer. Adjust 45% KOH solution meniscus to calibrating mark in nitrometer with digital readout meter. Record counter reading, R_1. (Counter reading should preferably lie between 500 and 1000 μL at this point. Vent control may be used to assist in arriving at this counter setting, if necessary.) Record syringe temperature, t_1, indicated on special scale thermometer. Add 2 min more to combustion portion of cycle by turning auxiliary timer to setting 3. (Once this is done, additional 2 min will be automatically programmed into each subsequent cycle.) Turn combustion cycle control to START. Let analyzer proceed through its cycle. After cycle is complete and combustion cycle control has entered STAND-BY section, readjust KOH meniscus to calibration mark with digital readout counter. Record new counter reading, R_2, and syringe temperature, t_2. Determine blank for instrument under same conditions as actual analysis except omit sample.

E. Calculations

(**a**) Record observed N_2 volume, $V_o = R_2 - R_1$, where V_o = observed N volume (μL), R_1 = initial counter reading, and R_2 = final counter reading.

(**b**) Determine corrected N_2 volume (in μL), $V_c = V_o - (V_b + V_t)$, where V_b = volume blank (μL), V_t = volume correction for temperature (μL) = $C_f(t_2 - t_1)$. C_f is obtained from Table **968.06A** (based on final counter reading); t_2 and t_1 are in °K.

(**c**) Determine corrected barometric pressure, $P_c = P_o - (P_b + P_v)$, where P_o = observed barometric pressure (mm Hg), P_b = barometric temperature correction (from Table **968.06B**), and P_v = pressure correction for vapor pressure of KOH solution (from Table **968.06C**).

Table 968.06A Volume Correction for Temperature Correction Factor (C_f) (μL/°K)[a]

Final Counter Reading, μL	C_f (Nitrometers with Check Value)
0	12
5000	29
10000	45
15000	62
20000	79
25000	95
30000	112
35000	129
40000	145
45000	162
50000	179

[a] Volume correction, $V_f = C_f(t_2 - t_1)$.

Table 968.06B Barometric Temperature Correction (P_b)

	P_0 (mm Hg)	
Temperature, °C	700—749	750—780
10	1.2	1.3
15	1.8	1.9
20	2.3	2.5
25	2.9	3.1
30	3.5	3.7
35	4.1	4.3

Table 968.06C Pressure Correction (P_v) for Vapor Pressure of KOH (for Practical Purposes, Temperature of KOH is Same as Syringe)

Temperature, °K	P_v, mm Hg
288	4.1
293	5.7
298	7.4
303	9.6
308	12.5
313	16.5

(*Note:* Empirical approximation of $(P_b + P_v) = 11.0$ will be satisfactorily accurate for P_o between 740 and 780 mm Hg and syringe temperature between 298 and 305°K.)

(**d**) Calculate % N = $(P_c \times V_c \times 0.0449)/(T \times W)$, where T = final syringe temperature in °K and W = sample weight in mg.

Example:

P_o = 750.1 mm Hg at 25°C; W = 148.91 mg

	Start	*Finish*
Counter readings, blank	500 μL	524 μL
Counter readings, sample	524	6955

t_1 = 302.7°K, t_2 = 303.0°K, V_o = 6955 – 524 = 6431 μL

$$V_c = 6431 - [24 + C_f(t_2 - t_1)] = 6431 - (24 + 35 \times 0.3) = 6396\ \mu L$$

$$P_c = 750.1 - (3.1 \times 9.6) = 737.4$$

$$\% N = (737.4 \times 6396 \times 0.04493)/(303.0 \times 148.91) = 4.69\%$$

(**e**) Calculate % protein = % N × 6.25, or % N × 5.70 in case of wheat grains.

Reference: JAOAC **51,** 766(1968).