4.1.10

AOAC Official Method 942.05
Ash of Animal Feed

First Action 1942
Final Action

Weigh 2 g test portion into porcelain crucible and place in temperature controlled furnace preheated to 600°C. Hold at this temperature 2 h. Transfer crucible directly to desiccator, cool, and weigh immediately, reporting percent ash to first decimal place.

$$\%\ (w/w)\ ash = \frac{\text{weight of test portion, g} - \text{weight loss on ashing, g}}{\text{weight of test portion, g}} \times 100$$

References: *JAOAC* **25,** 857(1942); **26,** 220(1943).

4.1.11

AOAC Official Method 994.12
Amino Acids in Feeds

Performic Acid Oxidation
with Acid Hydrolysis–Sodium Metabisulfite Method
First Action 1994
Final Action 1997

(Applicable to determination of amino acids [including methionine and cystine] in feeds. Not applicable to determination of tyrosine and tryptophan.)

See Tables **994.12A–E** for the results of the interlaboratory study supporting acceptance of method.

A. Principle

Performic acid oxidation is performed prior to hydrolysis to oxidize cystine and methionine to cysteic acid and methionine sulfone, respectively. Sodium metabisulfite is added to decompose performic acid. Amino acids are liberated from protein by hydrolysis with 6M HCl. Hydrolysates are diluted with sodium citrate buffer or neutralized, pH is adjusted to 2.20, and individual amino acid components are separated on ion-exchange chromatograph. Tyrosine is destroyed by oxidation. Tryptophan is destroyed by hydrolysis, so those amino acids cannot be determined.

B. Apparatus

(a) *Amino acid analyzer.*—Ion-exchange resin with ninhydrin post-column derivatization.

(b) *Analytical balance.*—Accurate to ±0.1 mg.

(c) *Balance.*—Top loading.

(d) *Bottle.*—50 mL; polyethylene.

(e) *Digestion tubes.*—Boiling flasks are suitable.

(f) *Digestion block.*—Heating mantle is suitable.

(g) *Filter units.*—0.22 μm (Millex GS, Millipore are suitable).

(h) *Magnetic stirring plate.*

(i) *pH meter.*—Calibrated with buffers of pH 2.0, 4.0, and 7.0.

(j) *Reflux condensers.*

(k) *Rotary evaporator.*

(l) *Vacuum flask.*—250 mL.

(m) *Glassware.*—Glass beakers, 250 and 1000 mL; Erlenmeyer flask, 150 mL; round-bottom evaporating flask, 1000 mL; graduated cylinders, 100, 500, and 1000 mL; volumetric flask, 1000 mL; volumetric pipets, 10 and 20 mL.

(n) *Sintered glass filter.*—Porosity 10–15 μm.

(o) *Ice bath.*

(p) *Syringes.*

C. Reagents

(a) *Formic acid.*—88%.

(b) *Hydrogen peroxide.*—30%.

(c) *Sodium metabisulfite.*

Table 994.12A Results of interlaboratory study for determination of amino acids in broiler finisher feed by sodium metabisulfite method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	44	1.17	0.032	2.74	0.099	8.46	0.090	0.277
Arginine	44	1.28	0.030	2.34	0.110	8.59	0.084	0.308
Aspartic acid	46	1.68	0.047	2.80	0.121	7.20	0.132	0.339
Cystine	38	0.32	0.010	3.13	0.036	11.25	0.028	0.101
Glutamic acid	44	3.25	0.052	1.60	0.226	6.95	0.146	0.632
Glycine	46	1.27	0.029	2.28	0.085	6.69	0.081	0.238
Histidine	34	0.50	0.020	4.00	0.099	19.80	0.056	0.277
Isoleucine	42	0.76	0.024	3.16	0.052	6.84	0.067	0.146
Leucine	46	1.66	0.044	2.65	0.105	6.33	0.123	0.294
Lysine	44	1.07	0.037	3.46	0.096	8.97	0.104	0.269
Methionine	38	0.53	0.006	1.13	0.040	7.55	0.017	0.112
Phenylalanine	44	0.87	0.038	4.37	0.127	14.60	0.106	0.356
Proline	36	1.39	0.044	3.17	0.124	8.92	0.123	0.347
Serine	44	0.94	0.044	4.68	0.127	13.51	0.123	0.356
Threonine	40	0.73	0.020	2.74	0.060	8.22	0.056	0.168
Valine	44	0.92	0.035	3.80	0.117	12.72	0.098	0.328

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12B Results of interlaboratory study for determination of amino acids in broiler starter feed by sodium metabisulfite method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	46	1.28	0.027	2.11	0.100	7.81	0.076	0.028
Arginine	46	1.57	0.042	2.68	0.129	8.22	0.118	0.361
Aspartic acid	40	2.29	0.035	1.53	0.137	5.98	0.098	0.384
Cystine	38	0.35	0.006	1.71	0.056	16.00	0.017	0.157
Glutamic acid	46	4.04	0.072	1.78	0.339	8.39	0.202	0.949
Glycine	46	1.27	0.034	2.68	0.090	7.09	0.095	0.252
Histidine	40	0.65	0.018	2.77	0.100	15.38	0.050	0.280
Isoleucine	46	0.95	0.019	2.00	0.098	10.32	0.053	0.274
Leucine	46	1.97	0.033	1.68	0.124	6.29	0.092	0.347
Lysine	46	1.35	0.032	2.37	0.122	9.04	0.090	0.342
Methionine	42	0.62	0.013	2.10	0.063	10.16	0.036	0.176
Phenylalanine	42	1.12	0.025	2.23	0.101	9.02	0.070	0.283
Proline	36	1.47	0.060	4.08	0.128	8.71	0.168	0.358
Serine	44	1.12	0.028	2.50	0.153	13.66	0.078	0.428
Threonine	44	1.12	0.024	2.73	0.087	9.89	0.067	0.244
Valine	40	1.11	0.019	1.71	0.098	8.83	0.053	0.274

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

(**d**) *DL-Norleucine.*—Crystals.

(**e**) *HCl.*—Concentrated.

(**f**) *NaOH.*—30% solution (30 g/100 mL).

(**g**) *Phenol.*—Crystals.

(**h**) *Thiodiglycol.*—98% solution.

(**i**) *Tri-sodium citrate dihydrate.*

(**j**) *pH buffer.*—pH 2.0, 4.0, and 7.0.

(**k**) *Amino acid standard kit.*—To calibrate amino acid analyzer; available from Aldrich Chemical Co., Inc., 1001 West Saint Paul Ave, Milwaukee, WI 53233, USA.

D. Preparation of Solutions

(**a**) *Sodium citrate buffer, pH 2.20.*—Weigh 19.60 g tri-sodium citrate dihydrate in 1000 mL beaker and dissolve in ca 800 mL H_2O. While stirring, add 10 mL 98% thiodiglycol solution and 15 mL con-

Table 994.12C Results of interlaboratory study for determination of amino acids in corn by sodium metabisulfite method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	44	0.61	0.009	1.48	0.049	8.03	0.025	0.137
Arginine	44	0.40	0.013	3.25	0.038	9.50	0.036	0.106
Aspartic acid	44	0.54	0.015	2.78	0.045	8.33	0.042	0.126
Cystine	38	0.18	0.007	3.89	0.025	13.89	0.020	0.070
Glutamic acid	40	1.51	0.036	2.38	0.094	6.23	0.101	0.263
Glycine	46	0.33	0.010	3.03	0.030	9.09	0.028	0.084
Histidine	42	0.27	0.019	7.04	0.063	23.33	0.053	0.176
Isoleucine	44	0.28	0.015	5.38	0.041	14.62	0.042	0.115
Leucine	44	0.99	0.019	1.92	0.069	6.97	0.053	0.193
Lysine	44	0.26	0.008	3.08	0.034	13.08	0.022	0.095
Methionine	42	0.18	0.010	5.56	0.021	11.67	0.028	0.059
Phenylalanine	40	0.38	0.009	2.37	0.073	19.21	0.025	0.204
Proline	34	0.73	0.019	2.60	0.044	6.03	0.053	0.123
Serine	40	0.39	0.007	1.79	0.040	10.26	0.020	0.112
Threonine	44	0.29	0.012	4.14	0.034	11.72	0.034	0.095
Valine	46	0.38	0.009	2.37	0.061	16.05	0.025	0.171

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12D Results of interlaboratory study for determination of amino acids in fishmeal by sodium metabisulfite method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	42	3.50	0.073	2.09	0.223	6.37	0.204	0.624
Arginine	46	3.40	0.117	3.00	0.280	7.18	0.328	0.784
Aspartic acid	46	5.22	0.108	2.07	0.327	6.26	0.302	0.916
Cystine	38	0.48	0.019	3.96	0.091	18.96	0.053	0.255
Glutamic acid	46	7.37	0.063	0.85	0.347	4.71	0.176	0.972
Glycine	46	3.84	0.059	1.54	0.215	5.60	0.265	0.602
Histidine	38	1.37	0.033	2.41	0.176	12.85	0.092	0.493
Isoleucine	46	2.32	0.049	2.11	0.238	10.26	0.137	0.666
Leucine	46	4.07	0.079	1.94	0.276	6.78	0.221	0.773
Lysine	44	4.22	0.117	2.77	0.335	7.94	0.328	0.938
Methionine	42	1.61	0.030	1.86	0.156	9.69	0.084	0.437
Phenylalanine	40	2.29	0.037	1.62	0.176	7.69	0.328	0.938
Proline	36	2.62	0.079	3.02	0.326	12.44	0.221	0.913
Serine	42	2.21	0.048	2.17	0.248	11.22	0.134	0.694
Threonine	44	2.28	0.081	3.55	0.244	10.70	0.227	0.683
Valine	44	2.78	0.063	2.27	0.311	11.19	0.176	0.871

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

centrated HCl. Transfer solution quantitatively into 1000 mL volumetric flask and dilute to mark with H_2O. Filter buffer solution through sintered glass filter, **B(n)**. Adjust pH to 2.20 with concentrated HCl or 2M NaOH.

(**b**) *6M HCl–phenol solution.*—Weigh 1 g phenol crystals into tared 1000 mL beaker. Dissolve crystals in 500 mL H_2O. While stirring, slowly add 500 mL concentrated HCl.

(**c**) *HCl solutions.*—(*1*) *1M HCl.*—Pour ca 800 mL H_2O into 1000 mL volumetric flask, and then add 83.3 mL concentrated HCl, using pipet. Dilute to the mark with H_2O and mix thoroughly. (*2*) *0.1M HCl.*—Pour ca 800 mL H_2O into 1000 mL volumetric flask, and then add 100 mL 1M HCl, using pipet. Dilute to the mark with H_2O and mix thoroughly.

(**d**) *NaOH solutions.*—(*1*) *7.5M NaOH.*—Weigh 300.0 g NaOH in tared 1000 mL beaker. (*2*) *2M NaOH.*—Weigh 80.0 g NaOH in tared

Table 994.12E Results of interlaboratory study for determination of amino acids in poultry meal by sodium metabisulfite method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	42	4.26	0.087	2.04	0.210	4.93	0.244	0.588
Arginine	46	4.35	0.144	3.31	0.420	9.66	0.403	1.176
Aspartic acid	44	4.92	0.132	2.68	0.376	7.64	0.370	1.053
Cystine	42	0.81	0.037	4.57	0.143	17.65	0.104	0.400
Glutamic acid	46	7.97	0.216	2.71	0.728	9.13	0.605	2.038
Glycine	38	6.90	0.085	1.23	0.286	4.14	0.238	0.801
Histidine	38	1.31	0.036	2.75	0.242	18.47	0.101	0.678
Isoleucine	46	2.24	0.060	2.68	0.261	11.65	0.168	0.731
Leucine	46	4.09	0.101	2.47	0.310	7.58	0.283	0.868
Lysine	46	3.63	0.112	3.09	0.360	9.92	0.314	1.008
Methionine	40	1.17	0.025	2.14	0.140	11.97	0.070	0.392
Phenylalanine	44	2.33	0.082	3.52	0.215	9.23	0.230	0.602
Proline	34	4.53	0.102	2.25	0.283	6.25	0.286	0.792
Serine	44	2.76	0.116	4.20	0.347	12.57	0.325	0.972
Threonine	44	2.32	0.073	3.15	0.212	9.14	0.204	0.594
Valine	44	2.82	0.090	3.19	0.361	12.80	0.252	1.011

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12F Results of interlaboratory study for determination of amino acids in broiler fnisher feed by hydrobromic acid method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r[a]	R[b]
Alanine	28	1.20	0.023	1.92	0.103	8.58	0.064	0.288
Arginine	30	1.22	0.032	2.62	0.170	13.93	0.090	0.476
Aspartic acid	26	1.75	0.032	1.83	0.065	3.71	0.090	0.182
Cystine	24	0.35	0.009	2.57	0.041	11.17	0.025	0.115
Glutamic acid	30	3.23	0.069	2.14	0.269	8.33	0.193	0.753
Glycine	30	1.30	0.040	3.08	0.125	9.62	0.112	0.350
Isoleucine	30	0.76	0.025	3.29	0.070	9.21	0.070	0.196
Leucine	26	1.69	0.033	1.95	0.086	5.09	0.092	0.241
Lysine	30	1.10	0.027	2.45	0.133	12.09	0.076	0.372
Methionine	24	0.54	0.011	2.04	0.033	6.11	0.031	0.092
Proline	24	1.41	0.025	1.77	0.200	14.18	0.070	0.560
Serine	30	0.94	0.043	4.57	0.142	15.11	0.120	0.398
Threonine	28	0.74	0.022	2.93	0.072	9.78	0.062	0.202
Valine	30	0.93	0.037	3.98	0.111	11.94	0.104	0.311

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

1000 mL beaker. Slowly dissolve pellets in each beaker in ca 600 mL H_2O. Cool solutions and transfer quantitatively to separate 1000 mL volumetric flasks. Dilute to mark with H_2O and mix thoroughly.

(**e**) *Norleucine standard solution.*—Accurately weigh 195–200 mg DL-norleucine crystals into tared 150 mL Erlenmeyer flask. Dissolve crystals with 100 mL 1M HCl. Transfer solution quantitatively into 1000 mL volumetric flask and dilute to mark with H_2O.

(**f**) *Performic acid reagent.*—Prepare in hood. Weigh 25 mg phenol crystals in 25 mL test tube; then add 0.5 mL 30% H_2O_2, using micropipet, and 4.5 mL 88% formic acid solution. Cover test tube with stopper, and let mixture stand 30 min at room temperature. After 30 min, place test tubes in ice bath and cool performic acid mixture for 15 min. Prepare reagent just before use.

E. Performic Acid Oxidation

Finely grind test sample to pass 0.25 mm sieve. Accurately weigh ca 100–1000 mg test portions to the nearest 0.1 mg (equivalent to ca 10 mg nitrogen content) into labeled digestion tubes.

Calculate approximate amount of test portion to use as follows:

$$W_s = \frac{1000}{N_s}$$

where N_s = nitrogen content of test portion, %; W_s = weight of test portion equivalent to 10 mg nitrogen content, mg.

Put magnetic stirrer into each tube and place digestion tubes in ice bath (0°C).

After both the performic acid and test portion have cooled at least 15 min, add 5 mL performic acid into digestion tube, cover all tubes with glass stoppers and stir 15 min on magnetic stirring plate.

Return digestion tubes to ice bath and let oxidize 16 h.

Remove glass stoppers and add ca 0.84 g sodium metabisulfite to decompose performic acid. Stir for 15 min to liberate SO_2.

F. Hydrolysis

Add 50 mL 6M HCl–phenol solution, **D(b)**, to test solution and briefly stir. Remove stirring bar using magnetic stirring rod, and rinse with small volume of 0.1M HCl into tube. Add 2–3 pieces of boiling chips to test solution.

Hydrolyze under reflux for 24 h at 110°–120°C using digestion block, **B(f)**. (*Caution*: Perform this step inside fume hood with adequate ventilation.)

Remove digestion tubes from heat and cool to room temperature. Add 20 mL norleucine standard solution, **D(e)**, to each hydrolysate using volumetric pipet. Mix solutions by swirling flasks. Proceed with (**a**) or (**b**) below. {*Note:* If low sodium concentration is required for chromatography, evaporate HCl carefully [perform step (**a**)]. If low sodium concentration is not required perform neutralization step, (**b**).}

(**a**) Filter hydrolysates through sintered glass filter into labeled 1000 mL evaporating flasks. Connect flasks to rotary evaporators, and evaporate under vacuum at 40°C to ca 5.0 mL. (*Note:* Do not let solution evaporate to dryness.) Remove flasks from evaporator. Add 50 mL sodium citrate buffer, **D(a)**, to evaporated test solution, mix well, and transfer into labeled 50 mL polyethylene bottle, **B(d)**. Proceed to **G**, or freeze until measurement.

(**b**) Filter hydrolysates into 250 mL vacuum flask, **B(l)**, through sintered glass filter, then transfer filtrate to 250 mL beaker. Place beaker in ice bath. Partly neutralize hydrolysates with ca 40 mL 7.5M NaOH, **D(d)**(*1*), while stirring. (*Note:* Temperature can not exceed 40°C.) Adjust pH to 2.20 using 2M NaOH, **D(d)**(*2*). Proceed to **G**.

G. Determination

Dilute aliquot of evaporated hydrolysate **F(a)** with sodium citrate buffer, **D(a)**, and adjust pH to 2.20 with 2M NaOH. When neutralized hydrolysates **F(b)** are used, dilute aliquot with H_2O. Filter through filter unit, **B(g)**, into autosampler tube and inject into analyzer. (*Note:* Volume of aliquot and dilution depends on response of analyzer.)

Calibrate amino acid analyzer with amino acid standard kit solution, **C(k)**, containing norleucine. Operate amino acid analyzer ac-

cording to manufacturer's specifications. Adjust analyzer conditions to ensure baseline separation of peaks. Minimum resolution between 2 peaks should be 90%.

H. Calculations

Calculate response factor (RF_{aa}) for each amino acid as follows:

$$RF_{aa} = \frac{P_n \times W_{aa}}{P_{aa} \times W_n}$$

where P_{aa} = peak area of amino acid; P_n = peak area of norleucine; W_{aa} = weight of amino acid, mg; W_n = weight of norleucine, mg.

Calculate internal standard (*IS*) factor as follows:

$$IS = W_n \times 2 \times 10^{-2}$$

where mg norleucine = norleucine content in 20 mL norleucine standard, **D(e)**.

Calculate amino acid (AA) content of the test sample as follows:

$$\text{AA, \%} = \frac{P_{aa} \times RF_{aa} \times IS \times 100}{P_n \times W_s}$$

where P_{aa} = peak area of amino acid; P_n = peak area of norleucine; W_s = weight of test portion, mg; RF_{aa} = amino acid response factor; *IS* = internal standard factor.

Performic Acid Oxidation with Acid Hydrolysis–Hydrobromic Acid Method

(Applicable to determination of amino acids [including methionine and cystine] in feeds. Not applicable to determination of phenylalanine, tyrosine, histidine, and tryptophan.)

See Tables **994.12F–J** for the results of the interlaboratory study supporting acceptance of method.

A. Principle

Performic acid oxidation is performed prior to hydrolysis to oxidize cystine and methionine to cysteic acid and methionine sulfone, respectively. Hydrobromic acid is added to decompose performic acid. Amino acids are liberated from protein by hydrolysis with 6M HCl. Hydrolysates are diluted with sodium citrate buffer and individual amino acid components are separated by ion-exchange chromatography. Tryptophan is destroyed by hydrolysis. Tyrosine, phenylalanine, and histidine are destroyed during oxidation process and by reaction with bromine, and cannot be accurately analyzed.

B. Apparatus

(a) *Amino acid analyzer.*—Ion-exchange resin with ninhydrin post-column derivatization.

(b) *Analytical balance.*—Accurate to ±0.1 mg.

(c) *Balance.*—Top loading.

(d) *Bottle.*—50 mL; polyethylene.

(e) *Digestion tubes.*—Boiling flasks are suitable.

(f) *Digestion block.*—Heating mantle is suitable.

(g) *Filter units.*—0.22 μm (Millex GS, Millipore are suitable).

(h) *Magnetic stirring plate.*

(i) *pH meter.*—Calibrated with buffers of pH 2.0, 4.0, and 7.0.

(j) *Reflux condensers.*

(k) *Rotary evaporator.*

(l) *Glassware.*—Glass beakers, 250 and 1000 mL; Erlenmeyer flask, 150 mL; round-bottom evaporating flask, 1000 mL; graduated cylinders, 100, 500, and 1000 mL; volumetric flask, 1000 mL; volumetric pipets, 10 and 20 mL.

(m) *Sintered glass filter.*—Porosity 10–15 μm.

(n) *Ice bath.*

(o) *Syringes.*

C. Reagents

(a) *Formic acid.*—88%.

(b) *Hydrobromic acid.*—48%.

(c) *Hydrogen peroxide.*—30%.

(d) *DL-Norleucine.*—Crystals.

(e) *HCl.*—Concentrated.

(f) *NaOH.*—30% solution (30 g/100 mL).

(g) *Phenol.*—Crystals.

(h) *Thiodiglycol.*—98% solution.

(i) *Tri-sodium citrate dihydrate.*

(j) *pH buffer.*—pH 2.0, 4.0, and 7.0.

(k) *Amino acid standard kit.*—To calibrate amino acid analyzer; available from Aldrich Chemical Co., Inc., 1001 West Saint Paul Ave, Milwaukee, WI 53233, USA.

D. Preparation of Solutions

(a) *Sodium citrate buffer, pH 2.20.*—Weigh 19.60 g tri-sodium citrate dihydrate in 1000 mL beaker and dissolve in ca 800 mL H_2O. While stirring, add 10 mL 98% thiodiglycol solution and 15 mL concentrated HCl. Transfer solution quantitatively into 1000 mL volumetric flask and dilute to mark with H_2O. Filter buffer solution through sintered glass filter, **B(m)**. Adjust pH to 2.20 with concentrated HCl or 2M NaOH.

(b) *6M HCl–phenol solution.*—Weigh 1 g phenol crystals into tared 1000 mL beaker. Dissolve crystals in 500 mL H_2O. While stirring, slowly add 500 mL concentrated HCl.

(c) *HCl solutions.*—(*1*) *1M HCl.*—Pour ca 800 mL H_2O into 1000 mL volumetric flask, and then add 83.3 mL concentrated HCl, using pipet. Dilute to the mark with H_2O and mix thoroughly. (*2*) *0.1M HCl.*—Pour ca 800 mL H_2O into 1000 mL volumetric flask, and then add 100 mL 1M HCl, using pipet. Dilute to the mark with H_2O and mix thoroughly.

(d) *Norleucine standard solution.*—Accurately weigh 195–200 mg DL-norleucine crystals into tared 150 mL Erlenmeyer flask. Dissolve crystals with 100 mL 1M HCl. Transfer solution quantitatively into 1000 mL volumetric flask and dilute to mark with H_2O.

(e) *Performic acid reagent.*—Prepare in hood. Weigh 25 mg phenol crystals in 25 mL test tube; then add 0.5 mL 30% H_2O_2, using micropipet, and 4.5 mL 88% formic acid solution. Cover test tube with stopper, and let mixture stand 30 min at room temperature. After 30 min, place test tubes in ice bath and cool performic acid mixture for 15 min. Prepare reagent just before use.

E. Performic Acid Oxidation

Finely grind test sample to pass 0.25 mm sieve. Accurately weigh ca 100–1000 mg test portions to the nearest 0.1 mg (equivalent to ca 10 mg nitrogen content) into labeled digestion tubes.

Calculate approximate amount of test portion to use as follows:

$$W_s = \frac{1000}{N_s}$$

Table 994.12G Results of interlaboratory study for determination of amino acids in broiler starter feed by hydrobromic acid method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	28	1.31	0.035	2.67	0.078	5.95	0.098	0.218
Arginine	30	1.51	0.034	2.25	0.206	13.64	0.095	0.577
Aspartic acid	28	2.36	0.077	3.26	0.204	8.64	0.216	0.571
Cystine	24	0.36	0.008	2.22	0.044	12.22	0.022	0.123
Glutamic acid	30	4.04	0.097	2.40	0.371	9.18	0.271	1.039
Glycine	30	1.29	0.040	3.10	0.130	10.08	0.112	0.364
Isoleucine	28	0.98	0.018	1.84	0.078	7.96	0.050	0.218
Leucine	26	2.03	0.025	1.23	0.093	4.58	0.070	0.260
Lysine	28	1.39	0.027	1.94	0.184	13.24	0.076	0.515
Methionine	24	0.63	0.008	1.27	0.028	4.44	0.022	0.078
Proline	22	1.43	0.029	2.03	0.131	9.16	0.081	0.367
Serine	28	1.14	0.037	3.25	0.193	16.93	0.104	0.540
Threonine	26	0.91	0.043	4.73	0.075	8.24	0.120	0.250
Valine	30	1.12	0.031	2.77	0.124	11.07	0.087	0.347

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

where N_s = nitrogen content of test portion, %; W_s = weight of test portion equivalent to 10 mg nitrogen content, mg.

Put magnetic stirrer into each tube and place digestion tubes in ice bath.

After both the performic acid and test portion have cooled at least 15 min, add 5 mL performic acid into digestion tube, cover all tubes with glass stoppers and stir 15 min on magnetic stirring plate.

Return digestion tubes to ice bath and let samples oxidize 16 h.

After oxidation, remove glass stoppers and decompose performic acid by adding 0.70 mL 48% hydrobromic acid, **C(b)**. Stir (held in ice bath) for 30 min to liberate bromine.

Transfer digestion tube to rotary evaporator, and swirl solution under vacuum at room temperature until color turns from bright orange to yellowish tint. Remove tube from evaporator and place on tube rack.

F. Hydrolysis

Add 50 mL 6M HCl–phenol solution, **D(b)**, to test solution and briefly stir. Remove stirring bar using magnetic stirring rod, and rinse with small volume of 0.1M HCl into tube. Add 2–3 pieces of boiling chips to test solution.

Hydrolyze under reflux for 24 h at 110–120°C using digestion block, **B(f)**. (*Caution:* Perform this step inside fume hood with adequate ventilation.)

Remove digestion tubes from heat and cool to room temperature.

Table 994.12H Results of interlaboratory study for determination of amino acids in corn by hydrobromic acid method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	30	0.61	0.014	2.30	0.046	7.54	0.039	0.129
Arginine	30	0.39	0.011	2.82	0.055	14.10	0.031	0.154
Aspartic acid	24	0.56	0.016	2.86	0.030	5.36	0.045	0.084
Cystine	24	0.19	0.006	3.16	0.021	11.05	0.017	0.059
Glutamic acid	30	1.49	0.038	2.55	0.116	7.79	0.106	0.325
Glycine	28	0.33	0.005	1.52	0.024	7.27	0.014	0.067
Isoleucine	30	0.29	0.008	2.76	0.033	11.38	0.022	0.098
Leucine	30	1.00	0.020	2.00	0.079	7.90	0.011	0.073
Lysine	30	0.26	0.008	3.08	0.035	13.46	0.092	0.174
Methionine	29	0.19	0.004	2.11	0.026	13.68	0.011	0.073
Proline	22	0.71	0.033	4.65	0.062	8.73	0.092	0.174
Serine	30	0.39	0.016	4.10	0.063	16.15	0.045	0.176
Threonine	24	0.30	0.005	1.67	0.014	4.67	0.014	0.039
Valine	29	0.39	0.011	2.82	0.053	13.59	0.031	0.148

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12I Results of interlaboratory study for determination of amino acids in fishmeal by hydrobromic acid method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r[a]	R[b]
Alanine	28	3.56	0.054	1.52	0.123	3.46	0.151	0.344
Arginine	30	3.24	0.087	2.69	0.421	12.99	0.244	0.179
Aspartic acid	30	5.26	0.194	3.69	0.437	8.31	0.543	1.224
Cystine	24	0.49	0.011	2.24	0.060	12.24	0.031	0.168
Glutamic acid	28	7.49	0.116	1.55	0.323	4.31	0.324	0.904
Glycine	26	3.91	0.052	1.33	0.149	3.81	0.146	0.417
Isoleucine	30	2.40	0.046	1.92	0.180	7.50	0.129	0.504
Leucine	24	4.15	0.061	1.47	0.152	3.66	0.171	0.426
Lysine	30	4.46	0.080	1.79	0.523	11.73	0.224	1.464
Methionine	24	1.63	0.041	2.52	0.081	4.97	0.115	0.227
Proline	24	2.54	0.083	3.27	0.393	15.47	0.232	1.100
Serine	30	2.23	0.055	2.47	0.292	13.09	0.154	0.818
Threonine	26	2.38	0.042	1.76	0.113	4.75	0.118	0.316
Valine	24	2.89	0.028	0.97	0.127	4.39	0.078	0.356

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12J Results of interlaboratory study for determination of amino acids in poultry meal by hydrobromic acid method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r[a]	R[b]
Alanine	28	4.28	0.074	1.73	0.193	4.51	0.207	0.540
Arginine	30	4.24	0.155	3.66	0.575	13.56	0.434	1.610
Aspartic acid	30	5.04	0.341	6.77	0.474	9.40	0.955	1.327
Cystine	24	0.82	0.025	3.05	0.098	11.95	0.070	0.274
Glutamic acid	28	8.05	0.251	3.12	0.497	6.17	0.703	1.392
Glycine	30	6.86	0.174	2.54	0.695	10.13	0.487	1.946
Isoleucine	26	2.31	0.020	0.87	0.173	7.49	0.056	0.484
Leucine	24	4.18	0.049	1.17	0.156	3.73	0.137	0.437
Lysine	28	3.72	0.052	1.40	0.356	9.57	0.146	0.997
Methionine	24	1.20	0.021	1.75	0.063	5.25	0.059	0.176
Proline	24	4.49	0.168	3.74	0.638	14.21	0.470	1.786
Serine	26	2.71	0.061	2.21	0.327	12.07	0.171	0.916
Threonine	24	2.38	0.038	1.60	0.119	5.00	0.106	0.333
Valine	30	2.85	0.090	3.16	0.302	10.60	0.252	0.846

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Add 20 mL norleucine standard solution, **D(d)**, to each test solution using volumetric pipet. Mix solution by swirling flask. Filter hydrolysates through sintered glass filter into labeled 1000 mL round-bottom evaporating flasks.

Evaporate hydrolysate at 60°C to dryness using rotary evaporator. Wash by adding ca 20 mL H_2O to hydrolysate and evaporate again. Repeat washing and evaporating steps 2×.

Remove flask from evaporator, add 50 ml sodium citrate buffer to hydrolysate and mix well. Transfer buffered hydrolysate into labeled 50 mL polyethylene bottles, **B(d)**. Proceed to **G**, or freeze until measurement.

G. Determination

Dilute aliquot of hydrolysate with sodium citrate buffer, **D(a)**, filter through 0.22 μm filter unit into autosampler tube, and inject into analyzer. (*Note:* Volume of aliquot and dilution depends on response of analyzer.)

Calibrate amino acid analyzer with amino acid standard kit solution, **C(k)**, containing norleucine. Operate analyzer according to manufacturer's specifications. Adjust analyzer conditions to ensure baseline separation of peaks. Minimum resolution between 2 peaks should be 90%.

H. Calculations

Proceed as in Performic Acid Oxidation with Acid Hydrolysis–Sodium Metabisulfite Method **H**.

Acid Hydrolysis Method

(Applicable to determination of amino acids in feeds except methionine, cystine, and tryptophan.)

Results of Interlaboratory Study:

See Tables **994.12K–O** for the results of the interlaboratory study supporting acceptance of method.

A. Principle

Amino acids are liberated from protein by hydrolysis with 6N HCl. Internal standard is added and HCl is evaporated. Hydrolysates are diluted with sodium citrate buffer and individual amino acid components are separated by ion-exchange chromatograph. Cystine and methionine are partially oxidized, and tryptophan is completely destroyed; therefore, they cannot by accurately quantified.

B. Apparatus

(a) *Amino acid analyzer.*—Ion-exchange resin with ninhydrin post-column derivatization.

(b) *Analytical balance.*—Accurate to ±0.1 mg.

(c) *Balance.*—Top loading.

(d) *Bottle.*—50 mL; polyethylene.

(e) *Digestion tubes.*—Boiling flasks are suitable.

(f) *Digestion block.*—Heating mantle is suitable.

(g) *Filter units.*—0.22 μm (Millex GS, Millipore are suitable).

(h) *pH meter.*—Calibrated with buffers of pH 2.0, 4.0, and 7.0.

(i) *Reflux condensers.*

(j) *Rotary evaporator.*

(k) *Glassware.*—Glass beakers, 250 and 1000 mL; Erlenmeyer flask, 150 mL; round-bottom evaporating flask, 1000 mL; graduated cylinders, 100, 500, and 1000 mL; volumetric flask, 1000 mL; volumetric pipets, 10 and 20 mL.

(l) *Sintered glass filter.*—Porosity 10–15 μm.

(m) *Syringes.*

C. Reagents

(a) *DL-Norleucine.*—Crystals.

(b) *HCl.*—Concentrated.

(c) *NaOH.*—30% solution (30 g/100 mL).

(d) *Phenol.*—Crystals.

(e) *Thiodiglycol.*—98% solution.

(f) *Tri-sodium citrate dihydrate.*

(g) *pH buffer.*—pH 2.0, 4.0, and 7.0.

(h) *Amino acid standard kit.*—To calibrate amino acid analyzer; available from Aldrich Chemical Co., Inc., 1001 West Saint Paul Ave, Milwakee, WI 53233.

D. Preparation of Solutions

(a) *Sodium citrate buffer, pH 2.20.*—Weigh 19.60 g tri-sodium citrate, dihydrate in 1000 mL beaker and dissolve in ca 800 mL H_2O. While stirring, add 10 mL 98% thiodiglycol solution, and 15 mL concentrated HCl. Transfer solution quantitatively into 1000 mL volumetric flask and dilute to mark with H_2O. Filter buffer solution through sintered glass filter, **B(l)**. Adjust pH to 2.20 with concentrated HCl or 2M NaOH.

(b) *6M HCl–phenol solution.*—Weigh 1 g phenol crystals into tared 1000 mL beaker. Dissolve crystals in 500 mL H_2O. While stirring, slowly add 500 mL concentrated HCl.

(c) *HCl solutions.*—(*1*) *1M HCl.*—Pour ca 800 mL H_2O into 1000 mL volumetric flask, and then add 83.3 mL concentrated HCl, using pipet. Dilute to the mark with H_2O and mix thoroughly. (*2*) *0.1M HCl.*—Pour ca 800 mL H_2O into 1000 mL volumetric flask, and then add 100 mL 1M HCl, using pipet. Dilute to the mark with H_2O and mix thoroughly.

(d) *NaOH solution.*—To make 2M NaOH: Weigh 80.0 g NaOH in tared 1000 mL beaker. Slowly dissolve pellets in beaker in ca 600 mL H_2O. Cool solution and transfer quantitatively to 1000 mL volumetric flask. Dilute to mark with H_2O and mix thoroughly.

(e) *Norleucine standard solution.*—Accurately weigh 195–200 mg DL-norleucine crystals into tared 150 mL Erlenmeyer flask. Dissolve crystals with 100 mL 1M HCl. Transfer solution quantitatively into 1000 mL volumetric flask and dilute to mark with H_2O.

E. Hydrolysis

Finely grind test sample to pass 0.25 mm sieve. Accurately weigh ca 100–1000 mg test portions to the nearest 0.1 mg (equivalent to ca 10 mg nitrogen content) into labeled digestion tubes.

Calculate approximate amount of test portion to use as follows:

$$W_s = \frac{1000}{N_s}$$

where N_s = nitrogen content of test portion, %; W_s = weight of test portion equivalent to 10 mg nitrogen content, mg.

Add 50 mL 6M HCl–phenol solution to test portion and briefly stir. Add 2–3 pieces of boiling chips to sample solution.

Hydrolyze under reflux for 24 h at 110°–120°C using digestion block, **B(f)**. (*Caution:* Perform this step inside fume hood with adequate ventilation.)

Table 994.12K Results of interlaboratory study for determination of amino acids in broiler finisher feed by acid hydrolysis method

Amino acids	N	Mean	s_r	RSD_R, %	s_R	RSD_R, %	r^a	R^b
Alanine	30	1.18	0.023	1.9	0.054	4.6	0.064	0.151
Arginine	28	1.25	0.029	2.3	0.12	9.3	0.081	0.336
Aspartic acid	28	1.67	0.061	3.7	0.12	7.3	0.171	0.336
Glutamic acid	24	3.24	0.037	1.1	0.14	4.3	0.104	0.392
Glycine	28	1.30	0.025	1.9	0.070	5.4	0.070	0.196
Histadine	30	0.50	0.028	5.6	0.054	11.0	0.078	0.151
Isoleucine	28	0.74	0.016	2.2	0.066	8.92	0.045	0.185
Leucine	26	1.66	0.020	1.2	0.069	4.2	0.056	0.193
Lysine	26	1.06	0.017	1.6	0.058	5.5	0.048	0.162
Phenylalanine	26	0.87	0.011	1.3	0.071	8.2	0.031	0.199
Proline	22	1.42	0.028	2.0	0.150	10.9	0.078	0.420
Serine	26	0.97	0.013	1.3	0.038	4.0	0.036	0.106
Threonine	26	0.74	0.014	1.9	0.043	5.9	0.039	0.120
Tyrosine	26	0.63	0.011	1.7	0.118	16.8	0.031	0.308
Valine	28	0.93	0.227	2.4	0.090	9.7	0.062	0.252

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12L Results of interlaboratory study for determination of amino acids in broiler starter feed by acid hydrolysis method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	30	1.29	0.36	2.8	0.068	5.3	0.101	0.190
Arginine	30	1.57	0.045	2.8	0.12	7.6	0.126	0.336
Aspartic acid	28	2.30	0.078	3.4	0.15	6.6	0.218	0.420
Glutamic acid	24	4.04	0.046	1.1	0.16	4.1	0.129	0.449
Glycine	30	1.29	0.018	1.4	0.076	5.9	0.050	0.213
Histamine	30	0.61	0.015	2.4	0.051	8.3	0.042	0.143
Isoleucine	30	0.96	0.034	3.6	0.10	10.6	0.095	0.280
Leucine	30	1.98	0.033	1.7	0.082	4.1	0.092	0.230
Lysine	30	1.35	0.027	2.0	0.096	7.1	0.076	0.269
Phenylalanine	28	1.11	0.021	1.9	0.081	7.3	0.059	0.227
Proline	22	1.50	0.043	2.9	0.095	6.4	0.120	0.266
Serine	26	1.17	0.020	1.7	0.088	7.5	0.056	0.246
Threonine	26	0.90	0.018	2.0	0.048	5.3	0.050	0.134
Tyrosine	26	0.84	0.012	1.4	0.045	5.3	0.034	0.126
Valine	28	1.11	0.029	2.6	0.12	10.5	0.081	0.336

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12M Results of interlaboratory study for determination of amino acids in corn by acid hydrolysis method

Amino acids	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	26	0.62	0.009	1.4	0.027	4.4	0.025	0.076
Arginine	26	0.37	0.010	2.5	0.033	8.4	0.028	0.092
Aspartic acid	28	0.54	0.018	3.3	0.036	6.6	0.050	0.101
Glutamic acid	26	1.54	0.020	1.3	0.11	6.9	0.056	0.308
Glycine	28	0.33	0.007	2.2	0.016	4.8	0.020	0.045
Histidine	28	0.24	0.005	2.3	0.020	8.4	0.014	0.056
Isoleucine	28	0.28	0.012	4.3	0.032	11.5	0.034	0.090
Leucine	28	0.99	0.014	1.4	0.044	4.4	0.039	0.123
Lysine	28	0.25	0.004	1.8	0.030	11.9	0.011	0.084
Phenylalanine	28	0.40	0.010	2.4	0.038	9.4	0.028	0.106
Proline	22	0.76	0.014	1.8	0.065	8.6	0.039	0.182
Serine	26	0.41	0.007	1.6	0.023	5.7	0.020	0.064
Threonine	28	0.30	0.015	5.1	0.029	9.6	0.042	0.081
Tyrosine	30	0.30	0.029	9.6	0.085	28.2	0.081	0.238
Valine	28	0.39	0.011	2.8	0.041	10.5	0.031	0.115

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12N Results of interlaboratory study for determination of amino acids in fishmeal by acid hydrolysis method

Amino acids	N	Mean	s_r	RSD_r, %	s_r	RSD_R, %	r^a	R^b
Alanine	30	3.49	0.079	2.3	0.20	5.7	0.221	0.560
Arginine	30	3.36	0.091	2.7	0.16	4.7	0.255	0.448
Aspartic acid	28	5.31	0.104	1.9	0.31	5.8	0.291	0.868
Glutamic acid	28	7.45	0.108	1.5	0.33	4.4	0.302	0.924
Glycine	26	3.88	0.031	0.8	0.205	5.3	0.087	0.574
Histidine	30	1.39	0.047	3.4	0.155	11.1	0.132	0.434
Isoleucine	30	2.35	0.071	3.0	0.240	10.2	0.199	0.672
Leucine	30	4.07	0.062	1.5	0.167	4.1	0.174	0.468
Lysine	28	4.25	0.073	1.7	0.268	6.3	0.204	0.750
Phenylalanine	30	2.24	0.063	2.8	0.175	7.8	0.176	0.490
Proline	24	2.65	0.061	2.3	0.280	10.6	0.171	0.784
Serine	30	2.25	0.054	0.145	0.145	6.4	0.151	0.406
Threonine	28	2.37	0.042	0.138	0.138	5.8	0.118	0.386
Tyrosine	30	1.85	0.04	0.168	0.168	9.1	0.134	0.470
Valine	30	2.82	0.087	0.300	0.300	10.6	0.244	0.840

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Table 994.12O Results of interlaboratory study for determination of amino acids in poultry meal by acid hydrolysis method

Amino acid	N	Mean	s_r	RSD_r, %	s_R	RSD_R, %	r^a	R^b
Alanine	30	4.26	0.098	2.3	0.193	4.5	0.274	0.540
Arginine	30	4.40	0.105	2.4	0.201	4.6	0.294	0.563
Aspartic acid	30	5.13	0.185	3.6	0.494	9.6	0.518	1.383
Glutamic acid	26	8.18	0.076	0.9	0.553	6.8	0.213	1.548
Glycine	30	6.98	0.147	2.1	0.444	6.4	0.412	1.243
Histidine	30	1.38	0.176	12.7	0.332	24.1	0.493	0.930
Isoleucine	28	2.30	0.039	1.7	0.147	6.4	0.109	0.412
Leucine	30	4.10	0.053	1.3	0.155	3.8	0.148	0.434
Lysine	28	3.67	0.093	2.5	0.274	7.5	0.260	0.767
Phenylalanine	30	2.33	0.059	2.5	0.187	8.0	0.165	2.436
Proline	26	4.78	0.141	3.0	0.568	11.9	0.395	1.590
Serine	28	2.86	0.076	2.7	0.145	5.1	0.213	0.406
Threonine	30	2.38	0.081	3.4	0.18	7.6	0.227	0.504
Tyrosine	30	1.78	0.047	2.6	0.224	12.6	0.132	0.627
Valine	30	2.90	0.11	3.8	0.295	10.2	0.308	0.826

[a] $r = 2.8 \times s_r$.

[b] $R = 2.8 \times s_R$.

Remove digestion tubes from heat and cool to room temperature. Add 20 mL norleucine standard solution, **D(e)**, to each test solution using volumetric pipet. Mix solutions by swirling flasks.

Filter hydrolysates through sintered glass filter into labeled 1000 mL round-bottom evaporating flasks. Connect flasks to rotary evaporators, and evaporate at 60°C to dryness. Wash by adding ca 20 mL H_2O and evaporate again. Repeat washing and evaporating steps 2×.

Remove flasks from evaporator. Add 50 mL sodium citrate buffer, **D(a)**, to evaporated hydrolysate, mix well, and transfer into labeled 50 mL polyethylene bottle, **B(d)**. Proceed to **F**, or freeze until ready for measurement.

F. Determination

Dilute aliquot of evaporated hydrolysate with sodium citrate buffer, **D(a)**, and adjust pH to 2.20 with 2M NaOH. When neutralized hydrolysates are used, dilute aliquot with H_2O. Filter through filter unit, **B(g)**, into autosampler tube and inject into analyzer. (*Note:* Volume of aliquot and dilution depends on response of analyzer.)

Calibrate amino acid analyzer with amino acid standard kit solution, **C(h)**, containing norleucine. Operate analyzer according to manufacturer's specifications. Adjust analyzer conditions to ensure baseline separation of peaks. Minimum resolution between two peaks should be 90%.

G. Calculations

Proceed as in Performic Acid Oxidation with Acid Hydrolysis–Sodium Metabisulfite Method **H**.

Reference: *J. AOAC Int.* **77**, 1362(1994).

Revised: March 1998

4.1.12

AOAC Official Method 999.12
Taurine in Pet Food
First Action 1999

(Applicable to the determination of 150–2000 mg total taurine/kg wet or dry cat or dog foods.)

See Table **999.12A** for the results of the interlaboratory study supporting acceptance of method.

A. Principle

The test portion is hydrolzyed with HCl and the extracted taurine reacted with dansyl chloride to form a fluorescent derivative determined by reverse-phase HPLC.

B. Apparatus

(**a**) *Liquid chromatograph.*—Gradient elution system, automatic sampler with 20 µL loop.

(**b**) *Fluorescence detector.*—Excitation wavelength, 298 nm; emission wavelength, 550 nm.

(**c**) *HPLC column.*—4 or 5 µm silica C8 or C18 reverse-phase column. (*Note:* A variety of specific column types may be accommodated with appropriate manipulation of the gradient elution profile.)

(**d**) *Filtration system.*—PVDF disposable syringe filters, 0.2 µm (Gelman Acrodisc, or equivalent).

(**e**) *Vortex mixer.*

C. Reagents

(**a**) *Dilute hydrochloric acid.*—6M. Add slowly 500 mL concentrated HCl to 500 mL water.

(**b**) *Sodium carbonate solution.*—0.2M. pH 9.7. Dissolve 21.2 g Na_2CO_3 in about 800 mL water. Adjust pH to 9.7 with 6M HCl, (**a**),