The cysteine, total sulfur amino acid, tyrosine, phenylalanine + tyrosine, and non-essential amino acid maintenance requirements of broiler breeders

R. D. Ekmay,[1] S. J. Mei,[2] N. K. Sakomura,[3] and C. N. Coon[4]

University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, Arkansas 72701

ABSTRACT Two hundred and fifty Cobb-Vantress broiler breeders were used to determine the maintenance requirement and efficiency of utilization of dietary Cys, Tyr, and non-essential amino acids (AA) in a 21-day experiment. The breeders were fed crystalline amino acid diets containing graded levels of Cys or Tyr representing 0, 10, 20, 30, and 40% of their suggested requirement level with all other amino acids maintained at 40% of their suggested requirement level. To determine the non-essential AA maintenance requirement, graded levels of non-essential AA were provided by glutamic acid to represent 12, 19, 26, 33, and 40% of the ideal level of glutamic acid with all other amino acids maintained at their maintenance requirement level. The total sulfur amino acid (TSAA) and Phe + Tyr requirements were calculated by combining Cys and Tyr results, respectively, with previously determined Met and Phe, respectively. The slope of Cys, Tyr, and non-essential AA accretion regression line indicated that 29% Cys, 24% TSAA, 21% Tyr, 20% Phe + Tyr, and 9% non-essential AA of crystalline amino acids were retained. The Cys requirement for zero protein accretion was calculated to be 30.48 mg/d or 17.006 mg/ kgBW$^{0.75}$/d or 75.426 mg/kgCP/d. The TSAA requirement for zero accretion was calculated to be 132.25 mg/b/d, 71.48 mg/kgBW$^{0.75}$/d, and 307.55 mg/kgCP/d. The Tyr requirement for zero protein accretion was calculated to be 65.907 mg/d or 37.233 mg/ kgBW$^{0.75}$/d or 175.566 mg/kgCP/d. The Phe + Tyr requirement for zero protein accretion was calculated to be 352.18 mg/b/d, 190.37 mg/kgBW$^{0.75}$/d, and 749.33 mg/kgCP/d. The non-essential AA requirement for zero protein accretion was calculated to be 3715.194 mg/d or 2003.155 mg/kgBW$^{0.75}$/d or 9452.954 mg/kgCP/d.

Key words: broiler breeders, TSAA, Phe + Tyr, non-essential amino acids, maintenance

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INTRODUCTION

The total amino acid (**AA**) requirement of a growing bird includes 2 components, a requirement for maintenance and a requirement for tissue protein accretion. There is evidence in pigs that the pattern of amino acids required for each of these is quite different, and the animal's total requirement must depend on the relative contributions of maintenance and tissue protein accretion to its total need (Fuller et al., 1989). Obligatory amino acid losses account for a portion of the maintenance requirements. In pigs, these may arise through increased losses in the digestive tract, increased oxidation, or by other routes; collectively these increased losses result in reduced efficiency of AA utilization (Fuller, 1994).

There have been a variety of methodologies utilized for the determination of maintenance requirements in broiler breeders (Gous et al., 1984, Nonis and Gous, 2008, Bonato et al., 2011). The maintenance AA requirement is normally defined as nitrogen equilibrium, the state in which nitrogen intake exactly equals the sum of nitrogen losses so that the nitrogen content of the body remains constant. Nonis and Gous (2008) and Bonato et al. (2011) defined nitrogen balance as the point in which the difference between nitrogen intake and nitrogen excretion is zero. Yet, nitrogen equilibrium also may be defined as the point at which there is no growth, no body protein accretion, or no AA accretion (Sakomura and Coon, 2003). There is strong evidence that using nitrogen balance (excretion) underestimates protein and AA maintenance requirements in cats (Laflamme and Hannah, 2013). Laflamme and Hannah (2013) state that nitrogen balance studies do not factor in changes to body composition or health caused by low protein intake, nor do they take into account prior nutritional status or current energy status.

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[1]Present address: Dow AgroSciences, LLC. Indianapolis, Indiana 46268.
[2]Present address: Winnepeg, Manitoba R3T 3W9.
[3]Present address: Department of Animal Science, College of Agrarian and Veterinary Sciences, University Estadual Paulista, Jaboticabal, São Paulo, Brazil, 14884-900.
[4]Correspondence author: ccoon@uark.edu

Another key limitation of attempts to quantify maintenance requirements is the reliance on the rooster to extrapolate the maintenance requirement of the hen. The objective of the present study is to determine the Cys, total sulfur amino acid (**TSAA**), Tyr, Phe + Tyr, and CP maintenance requirements of broiler breeder hens under a state of non-egg production using protein and amino acid accretion.

MATERIALS AND METHODS

General Procedures

All procedures were carried out in accordance with Animal Use Protocol No. 03008 for the experiment, which was approved by the University of Arkansas Institutional Animal Care and Use Committee. Two hundred and fifty Cobb-Vantress broiler breeder females, 22 wk of age, were selected for similar body weights (2222 ± 25 g) and housed in individual cages for a 21-day feeding period. The breeders were kept in environmentally controlled rooms (22°C) with a lighting schedule of 8 h light and 16 h dark.

The comparative slaughter technique (Wolynetz and Sibbald, 1987) was conducted to determine protein retention. Initial body composition data were obtained from 20 randomly selected birds (2167 ± 58 g) with similar body weight at the beginning of the experiment. Ten birds were randomly assigned to each experimental diet and fed 109 g/d for 21 d. Feed allotment was determined based on target BW as described in the Cobb-Vantress Breeder Management guide (2008). After 16 h of food deprivation, birds were killed by CO_2 asphyxiation and frozen at −4°C for subsequent analysis.

Cysteine and Tyrosine Determination

A chemically defined AA basal diet was formulated to meet all nutrient requirements (NRC, 1994) of broiler breeders with the exception of protein and AA. A purified basal diet was formulated to contain 3507 kcal (see Table 1) ME/kg, 6.78% protein equivalents from crystalline AA. Five purified diets with graded increases of Cys and Tyr, respectively, were mixed from the basal diet. Graded levels of Cys and Tyr, respectively, representing 0, 10, 20, 30, and 40% of their suggested level (NRC, 1994) were added with all other AA maintained at 40% of their suggested level. All diets were kept isonitrogenous (1.08% nitrogen) by adding additional amounts of glutamic acid. Each diet was offered (109 grams/bird/d) to 10 replicate birds. The composition of the basal diet is shown in Table 1.

Table 1. Basal diet and amino acid mixture for Cys and Tyr maintenance requirement.

Basal diet[1]		Amino acid mixture[2]	
Ingredients	%	Ingredients	%
Corn starch	Up to 100	L-Glutamate	6.93
Sucrose	17.00	L-Lysine	0.4
Corn oil	6.50	L-Methionine	0.09
Solka floc	4.00	L-Cysteine[5]	0.14
Dicalcium phosphate	2.22	L-Arginine	0.44
Limestone	1.74	L-Leucine	0.44
Magnesium oxide	0.10	L-Isoleucine	0.29
Potassium carbonate	0.80	L-Phenylalanine	0.30
Sodium chloride	0.30	L-Tyrosine[5]	0.23
Choline choride-60	0.26	L-Threonine	0.3
Sodium bicarbonate	0.80	L-Valine	0.33
Vitamin mixture[3]	0.13	L-Histidine-HCl-H_2O	0.13
Selenium premix	0.03	L-Tryptophan	0.07
Mineral mixture[4]	0.06		
Ethoxyquin	0.02		
Amino acid mixture[2]	9.74		

[1]Contained 3520 kcal ME/kg, 6.78% protein equivalents from crystalline amino acids.
[2]Amino acid mixture according to 40% of NRC (1994) recommendation.
[3]Provided per kilogram of diet: vitamin A, 13200IU; vitamin E, 66IU; vitamin D_3, 4950ICU; niacin, 74.25 mg; D-pantothenic acid, 33 mg; riboflavin, 19.8 mg; pyridoxine, 5000 mg; thiamine, 3.3 mg; menadione, 3.3 mg; folic acid, 3.3 mg; biotin, 0.33 mg; vitamin B_{12}, 0.0297 mg.
[4]Provided per kilogram of diet: manganese, 180 mg; zinc, 150.6 mg; iron, 20.16 mg; copper, 2.04 mg; iodine, 1.26 mg; selenium, 0.3 mg.
[5]Cys omitted from basal for Cys requirement determination and Tyr omitted from basal for Tyr requirement determination.

Non-Essential AA Determination

When determining non-essential AA (including Asp, Ser, Glu, Pro, Ala, and Gly) maintenance requirement, graded levels of glutamic acid were added to represent 12, 19, 26, 33, and 40% of its suggested level (NRC, 1994) with all other AA maintained at their maintenance requirement level (Sakomura and Coon, 2003). Each diet was offered (109 grams/bird/d) to 10 replicate birds. The basal diet formulation is shown in Table 2.

TSAA and Phe + Tyr Determination

The methionine level provided in the cysteine test diets (Table 1), as determined by Sakomura and Coon (2003), was added to the graded levels of cysteine to create the data set for TSAA determination. The phenylalanine level provided in the tyrosine test diets (Table 1) was added to the graded levels of tyrosine to create the data set for Phe + Tyr determination.

Analytical Procedures

All experimental birds that were euthanized for analysis were autoclaved to obtain homogeneous whole body samples. Birds were individually placed in trays, covered with foil and autoclaved at 120°C for 15 h. Then the birds were homogenized in a commercial blender and sub-samples were taken. The sub-samples were kept frozen at −20°C until dry matter and nitrogen analyses.

Autoclaved samples were freeze-dried by VIRTIS Advantage Instrument (SP Scientific, Gardiner, NY). Samples were weighed before and after drying to obtain dry-matter percentage. Whole-body nitrogen was analyzed on freeze-dried samples by Method 990.03 –

Table 2. Basal diet and amino acid mixture for the non-essential AA maintenance requirement.

Basal diet[1]		Amino acid mixture[2]	
Ingredients	%	Ingredients	%
Corn starch	Up to 100	L-Glutamate	8.05
Sucrose	17.00	L-Cysteine	0.14
Corn oil	6.50	L-Methionine	0.09
Solka floc	4.00	L-Tyrosine	0.23
Dicalcium phosphate	2.22	L-Lysine	0.21
Limestone	1.74	L-Arginine	0.35
Magnesium oxide	0.10	L-Leucine	0.19
Potassium carbonate	0.80	L-Isoleucine	0.15
Sodium chloride	0.30	L-Phenylalanine	0.30
Choline choride-60	0.26	L-Threonine	0.22
Sodium bicarbonate	0.80	L-Valine	0.18
Vitamin mixture[3]	0.13	L-Histidine-HCl-H_2O	0.10
Selenium premix	0.03	L-Tryptophan	0.02
Mineral mixture[4]	0.06		
Ethoxyquin	0.02		
Amino acid mixture[2]	10.42		

[1]Contained 3507 kcal ME/kg, 6.78% protein equivalents from crystalline amino acids.

[2]Amino acid mixture according to 40% of NRC (1994) recommendation.

[3]Provided per kilogram of diet: vitamin A, 13200IU; vitamin E, 66IU; vitamin D_3, 4950ICU; niacin, 74.25 mg; D-pantothenic acid, 33 mg; riboflavin, 19.8 mg; pyridoxine, 5000 mg; thiamine, 3.3 mg; menadione, 3.3 mg; folic acid, 3.3 mg; biotin, 0.33 mg; vitamin B_{12}, 0.0297 mg.

[4]Provided per kilogram of diet: manganese, 180 mg; zinc, 150.6 mg; iron, 20.16 mg; copper, 2.04 mg; iodine, 1.26 mg; selenium, 0.3 mg.

Combustion Method (AOAC, 1995). Whole-body AA composition was adopted from Sakomura and Coon (2003) with Cys, 1.92 g/100 g body protein; Tyr, 2.48 g/100 g body protein; and non-essential AA, 45.26 g/100 g body protein.

Statistical Analysis

The experiment was a one factor completely randomized design and the mean of 10 birds for each treatment was considered as the experimental unit. The general linear models procedure of SAS (SAS institute, version 8) was used to compute ANOVA. Microsoft Excel 2000 was used to derive linear regression equations. Six linear regression equations were determined for Cys, Tyr, and NEAA: protein (%N $\times$ 6.25) accretion (g/bird/d) vs. AA intake (mg/bird/d), protein accretion (g/kgBW$^{0.75}$/d) vs. AA intake (mg/kgBW$^{0.75}$/d), protein accretion (g/kgCP/d) vs. AA intake (mg/kgCP/d), AA accretion (g/bird/d) vs. AA intake (mg/bird/d), AA accretion (g/kgBW$^{0.75}$/d) vs. AA intake (mg/kgBW$^{0.75}$/d), and AA accretion (g/kgCP/d) vs. AA intake (mg/kgCP/d). Only 3 linear regression equations were generated for TSAA and Phe + Tyr: protein (%N $\times$ 6.25) accretion (g/bird/d) vs. AA intake (mg/bird/d), protein accretion (g/kgBW$^{0.75}$/d) vs. AA intake (mg/kgBW$^{0.75}$/d), and protein accretion (g/kgCP/d) vs. AA intake (mg/kgCP/d). A significant effect was determined when $P < 0.05$.

The AA requirements for maintenance (i.e., zero protein or AA accretion) were calculated by determining AA intake at zero protein or AA accretion.

The efficiency of AA utilization was determined by the slope of regression of AA accretion vs. AA intake.

RESULTS

Cysteine

Whole-body Cys increased linearly as dietary Cys levels increased from 0 to 40% of the ideal level (Table 3).

Using the linear regression equations that describe protein accretions (Y; g/b/d, g/kgBW$^{0.75}$/d and g/kgCP/d) as functions of Cys intake (X; mg/b/d, mg/kgBW$^{0.75}$/d and mg/kgCP/d), the estimated maintenance requirement for Cys were 30.48 mg/b/d, 17.006 mg/kgBW$^{0.75}$/d, and 75.426 mg/kgCP/d, respectively. In addition, the intercepts of these equations present the net daily loss of protein at 0.4633 g/b/d, 0.2653 g/kgBW$^{0.75}$/d, and 1.0258 g/kgCP/d, respectively.

Cys accretion (Y; mg/b/d, mg/kgBW$^{0.75}$/d and mg/kgCP/d) as a function of Cys intake (X; mg/b/d, mg/kgBW$^{0.75}$/d and mg/kgCP/d) was described by a straight line. Extrapolation of these linear regressions estimated maintenance requirements for Cys at 31.14 mg/b/d, 17.415 mg/kgBW$^{0.75}$/d, and 77.1 mg/kgCP/d. The slopes of these regression lines indicated that 27% of the Cys intake above maintenance was recovered as Cys in whole-body protein.

Cys accretion was a straight-line function of protein accretion. The linear regression equation describing this relationship was: Y = −0.2095 + 19.2X,

Table 3. Body weight gain, crude protein, and Cys accretion at graded levels of Cys intake.

Dietary	Feed intake	BW gain	Cys intake			AA accretion			Protein accretion		
Cys %	g/d	g/d/b	mg/d	mg/kgBW$^{0.75}$/d	mg/kgCP/d	mg/d	mg/kgBW$^{0.75}$/d	mg/kgCP/d	g/d	g/kgBW$^{0.75}$/d	g/kgCP/d
0	87.929	−0.833	0	0	0	−9.621	−5.199	−22.300	−0.490	−0.265	−1.136
0.03	88.147	1.704	26.444	14.689	64.412	0.432	0.044	−0.313	0.033	0.008	0.008
0.07	91.428	3.600	63.999	35.467	144.717	12.493	6.695	26.05	0.662	0.355	1.381
0.10	94.758	3.113	94.758	51.246	217.807	10.666	5.74	21.998	0.566	0.305	1.17
0.14	98.746	5.962	138.244	75.137	326.603	35.066	19.445	70.904	1.837	1.019	3.716
Pooled SEM	2.252	0.134	1.883	0.954	4.712	0.615	0.336	1.315	0.032	0.017	0.068
P-value						0.0098	0.0094	0.0079	0.0098	0.0094	0.0079

Table 4. Body weight gain and crude protein accretion at graded levels of TSAA intake.

Dietary	Dietary	Feed intake	BW gain	TSAA intake			Protein accretion		
Cys %	Met %	g/d	g/d/b	mg/d	mg/kgBW$^{0.75}$/d	mg/kgCP/d	g/d	g/kgBW$^{0.75}$/d	g/kgCP/d
0	0.090	87.929	−0.833	79.136	42.776	184.038	−0.490	−0.265	−1.136
0.03	0.090	88.147	1.704	105.777	57.177	245.992	0.033	0.008	0.008
0.07	0.090	91.428	3.600	146.284	79.073	340.196	0.662	0.355	1.381
0.1	0.090	94.758	3.113	180.039	97.319	418.696	0.566	0.305	1.170
0.14	0.090	98.746	5.962	227.115	122.765	528.175	1.837	1.019	3.716
Pooled SEM		2.252	0.134	1.883	0.954	4.712	0.324	0.189	0.744
P-value							0.0004	0.0004	0.0004

Table 5. Body weight gain, crude protein, and Tyr accretion at graded levels of Tyr intake.

Dietary	Feed intake	BW gain	Tyr intake			AA accretion			Protein accretion		
Tyr %	g/d	g/b/d	mg/d	mgBW$^{0.75}$/d	mg/kgCP/d	mg/d	mg/kgBW$^{0.75}$/d	mg/kgCP/d	g/d	g/kgBW$^{0.75}$/d	g/kgCP/d
0	90.225	−0.429	0	0	0	−17.82	−9.803	−43.603	−0.719	−0.395	−1.759
0.06	91.245	1.792	54.747	29.932	139.046	1.491	0.978	0.246	0.06	0.039	0.009
0.11	94.054	2.417	103.459	59.565	248.215	6.783	3.427	14.079	0.273	0.138	0.567
0.17	95.314	3.338	162.034	89.269	397.417	26.443	13.932	56.629	1.066	0.562	2.283
0.23	96.404	3.771	221.729	119.169	518.093	28.744	15.598	61.284	1.159	0.629	2.471
Pooled SEM	2.161	0.097	3.114	1.875	10.872	0.9	0.049	2.055	0.036	0.020	0.083
P-value						0.0061	0.0054	0.0038	0.0061	0.0054	0.0038

$r^2 = 1$ (Y = Cys accretion mg/b/d and X = protein accretion mg/b/d). Each one g increase in protein accretion resulted in an increase of 19.2 mg of Cys, indicating that Cys concentration in the whole-body protein accreted was a constant 1.92% at all levels of Cys intake. In addition, at zero protein accretion, birds lost 0.2095 mg/b/d, 0.1134 mg/kgBW$^{0.75}$/d, and 0.4713 mg/ kgCP/d of Cys.

TSAA

Using the linear regression equations that describe protein accretions (Y; g/b/d, g/kgBW$^{0.75}$/d and g/kgCP/d) as functions of TSAA intake (X; mg/b/d, mg/kgBW$^{0.75}$/d, and mg/kgCP/d), the estimated maintenance requirements for TSAA were 132.35 mg/b/d, 71.48 mg/kgBW$^{0.75}$/d, and 307.55 mg/kgCP/d (Table 4). In addition, the intercepts of these equations present the net daily loss of protein at 1.36 g/b/d, 0.74 g/kgBW$^{0.75}$/d, and 3.18 g/kgCP/d

Tyrosine

Results showed that whole-body Tyr increased linearly as dietary Tyr levels increased (Table 5).

Using the linear regression equations that describe protein accretions (Y; g/b/d, g/kgBW$^{0.75}$/d and g/kgCP/d) as a function of Tyr intake (X; mg/b/d, mg/kgBW$^{0.75}$/d, and mg/kgCP/d), the estimated maintenance requirements for Tyr were 65.907 mg/b/d, 37.233 mg/kgBW$^{0.75}$/d, and 175.566 mg/kgCP/d (Table 5). In addition, the intercepts of these equations represent the net daily loss of protein, 0.5668 g/b/d, 0.3202 g/kgBW$^{0.75}$/d, and 1.4572 g/kgCP/d.

Tyr accretion (Y; mg/b/d, mg/kgBW$^{0.75}$/d, and mg/kgCP/d) as a function of Tyr intake (X; mg/b/d, mg/kgBW$^{0.75}$/d, and mg/kgCP/d) were described by straight lines (Table 7). Extrapolation of these linear regressions resulted in the estimated maintenance requirements for Tyr of 65.706 mg/b/d, 37.053 mg/kgBW$^{0.75}$/d, and 174.746 mg/kgCP/d. The slopes of these regression lines indicated that 22% of the Tyr intake above maintenance was recovered as Tyr in whole-body protein.

Tyr accretion was a straight-line function of protein accretion. The linear regression equation describing this relationship was $Y = 0.0077 + 24.797X$, $r^2 = 1$ (Y = Tyr accretion mg/b/d and X = protein accretion mg/b/d). Each one g increase in protein accretion resulted in an increase of 24.797 mg of Tyr, indicating that Tyr concentration in the whole-body protein accreted was a constant 2.48% at all levels of Tyr intake. In addition, at zero protein accretion, birds accreted 0.0077 mg/bird/d, 0.0027 mg/kgBW$^{0.75}$/d, and 0.0147 mg/ kgCP/d of Tyr.

Phenylalanine + Tyrosine

Using the linear regression equations that describe protein accretions (Y; g/b/d, g/kgBW$^{0.75}$/d and g/kgCP/d) as a function of Phe+Tyr intake (X; mg/b/d, mg/kgBW$^{0.75}$/d, and mg/kgCP/d), the estimated maintenance requirements for Phe+Tyr were 352.18 mg/b/d, 190.37 mg/kgBW$^{0.75}$/d, and 749.33 mg/kgCP/d (Table 6). In addition, the intercepts of these equations represent the net daily

Table 6. Body weight gain and crude protein accretion at graded levels of Phe + Tyr intake.

Dietary Tyr %	Phe%	Feed intake g	BW gain g/b/d	Phe + Tyr intake mg/d	mg/kg$BW^{0.75}$/d	mg/kgCP/d	Protein accretion g/d	g/kg$BW^{0.75}$/d	g/kgCP/d
0.000	0.3	90.225	−0.429	270.676	146.311	575.906	−0.719	−0.395	−1.759
0.060	0.3	91.245	1.792	328.481	177.557	698.896	0.060	0.039	0.009
0.110	0.3	94.054	2.417	385.621	208.444	820.469	0.273	0.138	0.567
0.170	0.3	95.314	3.338	447.977	242.150	953.143	1.066	0.562	2.283
0.230	0.3	96.404	3.771	510.940	276.184	1087.107	1.159	0.629	2.471
Pooled SEM		2.161	0.097	3.114	1.875	10.872	0.130	0.070	0.300
P-value							<0.0001	<0.0001	<0.0001

Table 7. Body weight, crude protein, and non-essential AA accretion at graded levels of non-essential AA intake.

Non-essential AA level %	Feed intake g/d	BW gain g/d/b	Non-essential AA intake mg/d	mg/kg$BW^{0.75}$/d	mg/kgCP/d	AA accretion mg/d	mg/kg$BW^{0.75}$/d	mg/kgCP/d	Protein accretion g/d	g/kg$BW^{0.75}$/d	g/kgCP/d
0	89.332	−0.724	0	0	0	−17.820	−9.803	−43.603	−0.899	−0.476	−2.145
2.15	98.06	4.700	2108.3	1135.109	5107.6	1.491	0.978	0.246	−0.052	−0.031	−0.235
4.3	99.633	6.067	4284.233	2292.48	9859.463	6.783	3.427	14.079	0.122	0.059	0.169
6.45	97.513	7.176	6289.61	3304.693	14577.66	26.443	13.932	56.629	0.474	0.235	0.865
6.59	101.91	9.443	8784.68	4655.88	20204.76	28.744	15.598	61.284	0.515	0.273	1.126
Pooled SEM	1.397	0.122	88.852	43.843	414.836	3.13	1.85	7.30	0.04	0.021	0.088
P-value						0.0098	0.0095	0.0097	0.0098	0.0095	0.0097

loss of protein, 2.72 g/b/d, 1.47 g/kg$BW^{0.75}$/d, and 5.79 g/kgCP/d.

Non-Essential AA Maintenance Requirement

Whole-body protein increased linearly as dietary non-essential AA levels increased (Table 7).

Using the linear regression equations that describe protein accretions (Y; g/b/d, g/kg$BW^{0.75}$/d and g/kgCP/d) as functions of non-essential AA intake (X; mg/b/d, mg/kg$BW^{0.75}$/d, and mg/kgCP/d), estimated maintenance requirements for non-essential AA at 3,715.3 mg/b/d, 2,003.7 mg/kg$BW^{0.75}$/d, and 9,454.4 mg/kgCP/d. In addition, the intercepts of these equations represent the net daily loss of protein, 0.597 g/b/d, 0.335 g/kg$BW^{0.75}$/d, and 1.563 g/kgCP/d.

Non-essential AA accretion (Y; mg/b/d, mg/kg$BW^{0.75}$/d, and mg/kgCP/d) as a function of non-essential AA intake (X; mg/b/d, mg/kg$BW^{0.75}$/d, and mg/kgCP/d) were described by straight lines (Table 5). Extrapolation of these linear regressions estimated maintenance requirements for non-essential AA at 3,714.8 mg/b/d, 2,003.4 mg/kg$BW^{0.75}$/d, and 9,459.2 mg/kgCP/d. The slopes of these regression lines indicated that about 7% of the non-essential AA intake above maintenance was recovered as non-essential AA in whole-body protein.

Non-essential AA accretion was a straight-line function of protein accretion. The linear regression equation describing this relationship was $Y = -0.0472 + 452.6X$, $r^2 = 1$ (Y = non-essential AA accretion mg/b/d and X = protein accretion mg/b/d). Each one g increase in protein accretion resulted in an increase of 452.6 mg of non-essential AA, indicating that non-essential AA concentration in the whole-body protein accreted was a constant 45.26% at all levels of non-essential AA intake. In addition, at zero protein accretion, birds lost 0.0472 mg/bird/d, 0.0264 mg/kg$BW^{0.75}$/d, and 0.1087 mg/ kgCP/d of non-essential AA.

DISCUSSION

The data reported here are in agreement with other published data (Baker, 1996; Edwards and Baker, 1999a) showing constancy of utilization for Cys. Our data also agree with other published data showing that TSAA oxidation in chicks is greater than that of other AA (Muramatsu and Okumura, 1980). Total sulfur AA are the fast turnover AA and maintain the keratinous tissue of the animal. Leveille and Fisher (1960), based on a N balance study, estimated the Cys maintenance requirement for adult roosters to be 37 mg/kg/d and these authors suggested that the maintenance requirement for Cys was less than that for Met. Fisher (1998) calculated that the Met maintenance requirement for broiler breeders was 109 mg/b/d and Met + Cys maintenance requirement was 278 mg/b/d. The results implied that the maintenance requirement for Cys was 95 mg/b/d, which also indicated that the Cys maintenance requirement was less than that for Met. The results from the present study, however, place the Met + Cys maintenance requirement for broiler breeders at 132.25 mg/b/d, with the methionine requirement

Table 8. Summary of maintenance requirements for Cys, Met + Cys (TSAA), Tyr, Phe + Tyr, and non-essential amino acids (NEAA) based on zero protein accretion and zero amino acid accretion, respectively.

	Criteria (zero accretion)	Equation	mg/d	Equation	mg/kgBW$^{0.75}$/d	Equation	mg/kgCP/d
Cys	CP	0.0152x – 0.4633	30.5	0.0156x – 0.265	17.0	0.0136x – 1.0258	75.4
	AA	0.2924x – 9.105	31.1	0.2989x – 5.207	17.4	0.2617x – 20.177	77.1
TSAA	CP	0.0125x – 1.365	132.4	0.0126x – 0.738	71.5	0.0115x – 2.905	307.6
Tyr	CP	0.0086x – 0.567	65.9	0.0086x – 0.320	37.2	0.0083x – 1.457	175.6
	AA	0.2138x – 14.048	65.7	0.2142x – 7.937	37.1	0.2067x – 36.120	174.7
Phe + Tyr	CP	0.0079x – 2.720	352.2	0.0079x – 1.470	190.4	0.0079x – 5.787	749.3
NEAA	CP	0.1935x – 718.893	3,715.3	0.1913x – 383.277	2,003.7	0.1828x – 1728.150	9,454.4
	AA	0.0876x – 325.415	3714.8	0.0866x – 173.495	2,003.4	0.0827x – 782.277	9,459.2

set at 101.8 mg/b/d and the Cys requirement set at 30.48 mg/b/d (Table 8). Cave et al. (1990) reported the maintenance requirement of breeders for TSAA at 103.3 mg/kgBW/d. Edwards and Baker (1999a) using the same approach as the present study, fed birds from 5 to 95% of the ideal level and estimated the TSAA maintenance requirement for broilers 10 to 20 d old to be 15.3 mg/kgBW$^{0.75}$/d, and the efficiency of utilization was estimated to be 52%. Hruby et al. (1999) estimated the maintenance requirement of Met and Cys for broilers 10 to 21 days old to be 19 and 28 mg/kgBW$^{0.75}$/d, respectively. Bonato et al. (2011) reported a TSAA requirement of 59.5 mg/d or 25.5 mg/kgBW$^{0.75}$/d in Cobb roosters. Fuller et al. (1989) concluded that Cys could supply 79 to 82% of the TSAA needed for maintenance, which is not in agreement with the study reported herein. The efficiency of Met + Cys recovery in whole-body protein determined for broiler chicks (Edwards et al., 1999b) was superior to the efficiencies for the broiler breeders determined in this work. This is due, in part, to differences in age; young chicks at 2 wk old have greater potential for growth than broiler breeders at 23 wk of age and consequently, greater efficiency in whole-body protein deposition.

Leveille and Fisher (1960) estimated the Tyr maintenance requirement in the presence of 26 mg/kgBW of L-Phe for adult roosters to be 33 mg/kgBW. The author demonstrated that Tyr could spare about half the maintenance requirement and two-thirds of the minimum maintenance level for Phe. According to the results herein, the Phe + Tyr maintenance requirement for broiler breeders would be 352.18 mg/b/d, with the Phe requirement set at 229.58 mg/b/d (Table 8). The Tyr requirement was found to be 65.91 mg/b/d. However, the difference between the Phe+Tyr requirement and the Phe requirement indicates that the Tyr requirement would be 122.6 mg/b/d. The discrepancy may be explained by the Phe in the basal diet above the requirement being converted into Tyr, thus lowering the observed requirement for Tyr. Taking into consideration conversion of Phe into Tyr, the Tyr requirement from the present study then becomes 128.09 mg/b/d. Hruby et al. (1999) estimated the Phe and Tyr maintenance requirement for broilers 10 to 21 days old to be 27 and 20 mg/kgBW$^{0.75}$/d, respectively. Fisher (1998) calculated that Phe + Tyr requirement for broiler breeders to be 355 mg/b/d.

It would appear some AA play a more important role in maintenance than others (Kino and Okumura, 1986). Fuller et al. (1989) stated that Tyr plays a rather inferior role in the Phe/Tyr pair for maintenance requirements of pigs. The estimated Phe maintenance requirement was 3.4 times higher than the Tyr requirement. The data reported herein indicate that Phe also plays a superior role in the Phe/Tyr pair for maintenance in broiler breeders.

Since the chemical composition of a broiler breeder's body changes with age and with the type of diet, it was postulated that defining the AA maintenance requirement on crude protein body weight instead of metabolic body weight would be more logical. Also AA requirement per kg CP body mass should be more specific than metabolic BW since AA utilization would primarily replace tissue AA, not other types of body composition. The maintenance AA requirements based on crude protein weight (kg) would allow making comparisons between maintenance requirements of young and older broilers. Expressed as mg/kgCP/d, the Tyr requirement for breeders is 175.566 mg/kgCP/d, which was similar to the estimation of 167.82 mg/kgCP/d for broilers (Hruby et al., 1999).

The non-essential AA requirement for broiler breeders was calculated to be 4057.66 mg/d, which is equivalent to 386.3 mg N/d (Table 8). According to Sakomura and Coon (2003) and the results from this study, the total maintenance requirement of essential AA nitrogen for broiler breeders was calculated to be 287.72 mg N/d. Thus, the ratio of essential AA nitrogen to non-essential AA nitrogen maintenance requirement was calculated to be 74.48%. The ratio indicated that non-essential AA played an important role in fulfilling breeder maintenance AA requirements. According to the NRC (1994), the total essential + non-essential AA requirement for broiler breeder is 19,500 mg/d, while the total essential AA nitrogen requirement is 1,145.35 mg N/d; the calculated non-essential AA nitrogen requirement is 1,578.15 mg N/d. The ratio of essential AA to non-essential AA nitrogen requirement was calculated to be 72.58%. This implied that non-essential AA played an important role in AA maintenance + production requirement. Moran et al. (1983) estimated the maintenance nitrogen requirement of the turkey breeder hen to be 257 mg N/kgBW/d. Leveille and Fisher (1958) reported, using glycine as non-essential amino acid nitrogen to estimate the non-protein depleted nitrogen maintenance requirement for adult roosters, a nitrogen maintenance requirement of 280 mg N/kgBW/d. These results are lower than the data reported herein. Emmans (1987) suggested a universal requirement for scaled ideal protein requirement for growing birds of 8,000 mg/kgCP$^{0.73}$, which is similar with the study reported herein.

The maintenance requirements present within do not show consistency across methodologies. In some instances, the results of the current methodology are in agreement with nitrogen balance studies and in some instances are in agreement with protein accretion methodologies. Nevertheless, these results are valuable in that they were determined using hens, and, thus, eliminate a possible variable.

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