Ideal Ratios of Isoleucine, Methionine, Methionine Plus Cystine, Threonine, Tryptophan, and Valine Relative to Lysine for White Leghorn-Type Laying Hens of Twenty-Eight to Thirty-Four Weeks of Age

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ABSTRACT Seven separate experiments were conducted with Hy-Line W-36 hens to determine the ideal ratio of Arg, Ile, Met, Met+Cys, Thr, Trp, and Val relative to Lys for maximal egg mass. The experiments were conducted simultaneously and were each designed as a randomized complete block design with 60 experimental units (each consisting of 1 cage with 2 hens) and 5 dietary treatments. The 35 assay diets were made from a common basal diet (2,987 kcal/kg of ME; 12.3% CP; 4.06% Ca, 0.47% nonphytate P), formulated using corn, soybean meal, and meat and bone meal. The true digestible amino acid contents in the basal diet were determined using the precision-fed assay with adult cecectomized roosters. Crystalline L-Arg (free base), L-Ile, L-Lys•HCl, DL-Met, L-Thr, L-Trp, and L-Val (considered 100% true digestible) were added to the basal diet at the expense of cornstarch to make the respective assayed amino acid first limiting and to yield 5 graded inclusions of the assayed amino acid. Hens were fed the assay diets from 26 to 34 wk of age, with the first 2 wk considered a depletion period. Egg production was recorded daily and egg weight was determined weekly on eggs collected over 48 h; egg mass was calculated as egg production × egg weight. The requirement for each amino acid was determined using the broken-line regression method. Consumption of Arg did not affect egg mass, thus a requirement could not be determined. The true digestible amino acid requirements used to calculate the ideal amino acid ratio for maximum egg mass were 426 mg/d of Ile, 538 mg/d of Lys, 253 mg/d of Met, 506 mg/d of Met+Cys, 414 mg/d of Thr, 120 mg/d of Trp, and 501 mg/d of Val. The ideal amino acid ratio for maximum egg mass was Ile 79%, Met 47%, Met+Cys 94%, Thr 77%, Trp 22%, and Val 93% on a true digestible basis relative to Lys. The ideal Met and Met+Cys ratios were verified in an ensuing identical experiment with 52- to 58-wk-old hens.

Key words: egg mass, ideal amino acid profile, laying hen

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INTRODUCTION

Amino acid (**AA**) requirements for laying hens are published by the NRC (1994). However, the experiments upon which these requirements are based are dated and do not account for the genetic progress of laying hens that has occurred over the last 15 yr. Amino acid requirements have been reported since the publication of the NRC (1994) (e.g., Schutte et al., 1994; Waldroup and Hellwig, 1995; Ishibashi et al., 1998; Schutte and Smink, 1998; Coon and Zhang, 1999; Russell and Harms, 1999; Harms and Russell, 2000a,b, 2001; Faria et al., 2002). However, these experiments have been conducted for a single AA at a time and were performed under different experimental conditions with different basal diets, genetic lines, feed consumption rates, dietary energy contents, feedstuffs, ambient temperature, cage space, and ages of laying hens, all of which influence the AA requirements (Leeson and Summers, 2001; Baker et al., 2002; D'Mello, 2003). Because multiple factors affect AA requirements, those determined under experimental conditions may not be applicable under field conditions and estimations of AA requirements should instead be based on the ideal AA profile (Baker and Han, 1994; Baker et al., 2002). The ideal AA profile employs the concept that, whereas absolute AA requirements change due to genetic or environmental factors, the ratios among them are only slightly affected. Thus, once the ideal AA profile has been determined, the requirement for a single AA (i.e., Lys) can be determined experimentally for a given field situation and the requirements for all other AA calculated from the ideal ratios. Such an approach has been adopted by the swine industry (NRC, 1998) and is finding use in the broiler industry as well (Emmert and Baker, 1997; Mack et al., 1999; Baker et al., 2002; Baker, 2003; Dari et al., 2005).

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Table 1. Ingredient and chemical composition of the control and basal diets (as-is basis)

	Experiment 1		Experiment 2	
Item	Control	Basal %	Control	Basal
Ingredient				
Cornstarch[1]	—	5.00	—	5.00
Corn	54.50	70.40	58.30	72.47
Soybean meal (48% CP)	24.02	6.00	22.00	6.00
Meat and bone meal (50% CP)	5.00	5.00	2.50	2.50
Vegetable oil	5.73	2.00	4.62	1.00
DL-Met	0.28	—	0.19	—
Potassium carbonate	0.04	0.83	—	0.56
Dicalcium phosphate	0.74	0.94	0.90	1.05
Calcium carbonate[2]	8.83	8.73	10.59	10.52
Sodium chloride (iodized)	0.21	0.45	0.25	0.25
Trace mineral premix[3]	0.30	0.30	0.30	0.30
Vitamin premix[4]	0.35	0.35	0.35	0.35
Total	100.00	100.00	100.00	100.00
Analyzed composition[5]				
CP	16.67	12.28	16.05	9.60
Arg (total)	1.08	0.75	1.08	0.55
Gly+Ser (total)	1.69	1.30	1.56	1.07
His (total)	0.45	0.29	0.42	0.25
Ile (total)	0.63	0.41	0.65	0.35
Leu (total)	1.37	1.01	1.38	0.95
Lys (total)	0.88	0.55	0.88	0.41
Met (total)	0.51	0.20	0.44	0.15
TSAA (total)	0.75	0.38	0.68	0.30
Phe (total)	0.76	0.47	0.78	0.45
Thr (total)	0.62	0.43	0.61	0.34
Trp (total)	0.19	0.10	0.19	0.11
Val (total)	0.76	0.53	0.74	0.44
Calculated composition				
ME_n, kcal/kg	2,987	2,987	2,909	2,909
Ether extract	8.43	5.20	7.21	4.04
Linoleic acid	3.38	2.31	3.69	2.13
Ca	4.06	4.06	4.50	4.50
P (nonphytate)	0.47	0.47	0.38	0.38
K	0.74	0.74	0.66	0.66
Na	0.22	0.32	0.22	0.22
Cl	0.33	0.47	0.34	0.34
K + Na − Cl, mEq/kg	193	193	170	170

[1]Cornstarch was partially replaced with crystalline amino acids and potassium carbonate to create the assay diets.

[2]Calcium carbonate was supplied as a 1:1 mix of fine and coarse particles (mean diameter 0.14 and 2.27 mm, respectively).

[3]Supplied per kilogram of diet: manganese (manganese sulfate), 70 mg; zinc (zinc oxide), 90 mg; iron (ferrous sulfate), 60 mg; copper (cupric sulfate), 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

[4]Supplied per kilogram of diet: vitamin A, 9,261 IU; vitamin D_3, 3,087 IU; vitamin E, 15.4 IU; vitamin B_{12}, 12.3 μg; vitamin K, 1.5 mg; riboflavin, 6.2 mg; D-pantothenic acid, 10.8 mg; niacin, 30.9 mg; choline, 385.9 mg; folic acid 0.5 mg; biotin, 46.3 μg.

[5]Analyses performed in duplicate.

Unlike the NRC for swine (1998), neither the NRC for poultry (1994) nor the Dutch Centraal Veevoederbureau (**CVB**) (1996) use the ideal AA profile to determine the AA requirements for laying hens, but report empirically determined AA requirements. These AA requirements can be used to calculate ideal AA profiles for laying hens; however, these profiles were estimated from data compiled from a variety of experiments and, thus, were influenced by genetics and environmental factors as mentioned previously. Coon and Zhang (1999) conducted 5 separate experiments to determine the AA requirements of laying hens and reported the ideal AA profile from averages of the 5 experiments. Although better than the ideal AA profile calculated from NRC (1994) or CVB (1996) recommendations, these experiments were still

Table 2. True digestibility (%) of amino acids in the corn-soybean meal based basal diets[1]

Amino acid	Experiment 1	Experiment 2
Arg	92.0	92.6
Cys	88.0	79.4
His	88.3	85.2
Ile	89.5	88.5
Leu	93.1	91.0
Lys	91.9	86.8
Met	93.9	91.9
Phe	91.8	89.9
Thr	88.5	81.7
Trp	93.7	92.3
Val	88.7	83.6

[1]Values are means of 5 observations from the cecectomized rooster assay.

Table 3. Addition of crystalline amino acids (AA) in experiment 1, replacing cornstarch in the basal diet[1]

Assay	AA added	AA diet				
		1[2]	2	3	4	5
		%				
AA addition						
Arg	L-Arg, free base	—	0.062	0.123	0.185	0.246
Ile	l-Ile	—	0.141	0.283	0.424	0.566
Lys	L-Lys•HCl	—	0.232	0.464	0.695	0.927
Met	DL-Met	—	0.222	0.443	0.666	0.887
Thr	L-Thr	—	0.075	0.150	0.225	0.300
Trp	L-Trp	—	0.041	0.082	0.121	0.162
Val	L-Val	—	0.140	0.280	0.420	0.560
Dietary true digestible AA content[3]						
Arg	Arg	0.69	0.75	0.81	0.87	0.93
Ile	Ile	0.37	0.50	0.64	0.78	0.92
Lys	Lys	0.51	0.69	0.87	1.05	1.24
Met	Met	0.19	0.41	0.63	0.85	1.07
Met+Cys[4]	Met+Cys	0.35	0.57	0.78	1.01	1.22
Thr	Thr	0.38	0.45	0.53	0.60	0.67
Trp	Trp	0.09	0.13	0.17	0.21	0.25
Val	Val	0.47	0.61	0.74	0.88	1.02

[1]All AA, except the assayed AA, were included at diet 3, diet 4, diet 4, diet 5, and diet 5 levels for diets 1 to 5, respectively, to avoid antagonisms among AA and to ensure that the assayed AA was first-limiting in the particular assay diet.

[2]Basal diet.

[3]Calculated from the analyzed total AA content of the basal diet multiplied by the analyzed digestibility coefficient plus the inclusion of crystalline AA (the latter assumed 100% digestible).

[4]The responses to TSAA were evaluated in the Met assay; graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet.

performed under different experimental conditions with different basal diets, ages, and genetic lines of hens. To ensure a valid measurement of the ideal AA profile, the same basal diet, the same genetic line, and the same assay period should be used in all assays of AA requirements (Baker, 2003). Hence, the objective of experiment (**Exp.**) 1 was to investigate responses of laying hens, 28 to 34 wk of age, to graded dietary inclusions of the essential AA Arg, Ile, Lys, Met, Thr, Trp, and Val, to determine the ideal AA ratios of the assayed AA relative to Lys. In Exp. 2, the objective was to confirm the Lys, Met, and TSAA requirements (and, therefore, the ideal Met:Lys and TSAA:Lys ratios) determined in Exp. 1.

MATERIALS AND METHODS

In Exp. 1, the responses to Arg, Ile, Lys, Met, TSAA, Thr, Trp, and Val were investigated at the same time using hens of the same age and genetic line, fed the same basal diet (mixed in 2 large batches to minimize variation in nutrient contents among assay diets), and housed in the same barn. In effect, Exp. 1 consisted of 7 individual

Table 4. Addition of crystalline amino acids (AA) in experiment 2, replacing cornstarch in the basal diet[1]

Assay	AA	Assay diet				
		1[2]	2	3	4	5
		%				
AA addition						
Lys	L-Lys•HCl	—	0.194	0.389	0.583	0.777
Met	DL-Met	—	0.158	0.316	0.474	0.632
True digestible AA content[3]						
Lys	Lys	0.34	0.46	0.59	0.72	0.84
Met	Met	0.13	0.25	0.37	0.48	0.60
Met+Cys[4]	Met+Cys	0.36	0.47	0.59	0.71	0.83

[1]All AA, except the assayed AA, were included at diet 3, diet 4, diet 4, diet 5, and diet 5 levels for diets 1 to 5, respectively, to avoid antagonisms among AA and to ensure that the assayed AA was first-limiting in the particular assay diet.

[2]Basal diet.

[3]Calculated from the analyzed total AA content of the basal diet multiplied by the analyzed digestibility coefficient plus the inclusion of crystalline AA (the latter assumed 100% digestible).

[4]The responses to TSAA were evaluated in the Met assay; graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet.

dose-response assays (i.e., 1 for each AA assayed; Met and TSAA were analyzed in the same assay) conducted simultaneously in the same barn. Each of these 7 assays was a randomized complete block design with 12 blocks and 5 graded levels of the AA assayed. Experiment 2 was conducted 4 mo after Exp. 1 with the same hens to confirm the observed Lys, Met, and TSAA requirements from Exp. 1 (i.e., 2 assays, each a randomized complete block design with 12 blocks with 5 graded levels of either Lys or Met). All procedures relating to the use of live animals were approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals and Housing

A total of 1,008 white single-comb Leghorn-type hens (Hy-Line W-36, Hy-Line International, Des Moines, IA), 18 wk of age, were placed in a windowless fan-ventilated room. Hens were housed 2 per cage, corresponding to 619 cm^2 per hen, in wire-bottomed cages (Chore-Time, Milford, IN), each equipped with a plastic self-feeder and a nipple drinker. Before Exp. 1, all hens were given free access to corn and soybean meal diets following recommendations listed in the W-36 Commercial Management Guide (Hy-Line International). The photoperiod was increased incrementally to 16L:8D at 26 wk of age in accordance with the W-36 Commercial Management Guide. A corn-soybean meal based diet, exceeding the NRC (1994) nutrient recommendations, was fed in the period between Exp. 1 and 2.

Experimental Diets

Before formulation of the assay diets, all protein-supplying ingredients were analyzed for contents of individual AA by ion-exchange chromatography (Llames and Fontaine, 1994). An industry-type control diet (Table 1) was formulated using corn, soybean meal, and meat and bone meal, a calculated ME_n content similar to that of the AA-deficient basal diet, and formulated to meet or exceed nutrient recommendations by the NRC (1994). In addition, a basal diet was formulated to meet or exceed nutrient recommendations by the NRC (1994), except for the 7 assayed AA, using feed ingredients typically used in commercial diets (i.e., corn, soybean meal, and meat and bone meal). In Exp. 1, the basal diet was mixed in 2 separate 2,722-kg batches in a horizontal mixer at a commercial feed mill and bagged in 22.6-kg bags according to batch number. In Exp. 2, a basal diet was mixed in a vertical mixer in 3 separate 500-kg batches at the Iowa State University Poultry Science Research Center. Representative samples of the basal-diet batches were pooled within experiment and analyzed for AA content by ion-exchange chromatography (Llames and Fontaine, 1994) and for true AA digestibility (Table 2) by the cecectomized rooster assay at the Department of Poultry Science, University of Georgia, Athens, GA (Lumpkins and Batal, 2005). The crystalline AA added to the basal diet were assumed to be 100% true digestible (Izquierdo et al., 1988; Chung and Baker, 1992).

The 35 assay diets in Exp. 1 were formulated with equal parts of each of the 2 basal-diet batches plus a mixture of cornstarch, K_2CO_3 (to maintain a similar dietary electrolyte balance among all diets), and crystalline AA to create 5 equally spaced graded inclusions of each of the 7 assayed AA, such that diet 3 provided the assayed AA at the estimated requirement (Table 3). Hence, K_2CO_3 and crystalline AA partially replaced cornstarch in the basal diet. To avoid antagonisms and imbalances among AA and to ensure that the assayed AA was first-limiting in the particular assay diet, the basal diet in Exp. 1 was fortified with all AA under investigation—except the one assayed—to contain, on a true digestible basis, 0.81% Arg, 0.64% Ile, 0.87% Lys, 0.63% Met, 0.78% Met+Cys, 0.53% Thr, 0.17% Trp, and 0.74% Val in assay diet 1; 0.87% Arg, 0.78% Ile, 1.05% Lys, 0.85% Met, 1.01% Met+Cys, 0.60% Thr, 0.21% Trp, and 0.88% Val in assay diets 2 and 3; and 0.93% Arg, 0.92% Ile, 1.24% Lys, 1.07% Met, 1.22% Met+Cys, 0.67% Thr, 0.25% Trp, and 1.02% Val in assay diets 4 and 5. In other words, crystalline AA, other than the one assayed, were included in diet 1, 2, 3, 4, and 5 to supply the true digestible AA concentration found in diet 3, 4, 4, 5, and 5, respectively (see Table 3). In Exp. 2, only the responses to Lys and Met were determined; thus, there were 10 assay diets formulated with equal parts of each of the 3 basal-diet batches as described for Exp. 1 (Table 4).

In both experiments, the individual assay diets were mixed by first premixing the crystalline AA, K_2CO_3, and cornstarch with a small amount of basal diet in a Hobart mixer; the premix and the remainder of the basal diet (equal amounts from the respective batches) were then mixed in a 340-L horizontal ribbon mixer and fed in mash form. Representative samples of all assay diets were analyzed for individual AA by ion-exchange chromatography; the analyses agreed well with the calculated values shown in Tables 3 and 4 (data not shown) and only calculated values are tabulated.

Data Collection

Hens were offered free access to the experimental diets from 26 to 34 wk of age (Exp. 1) or from 50 to 58 wk of age (Exp. 2), with the first 2 wk of each experiment considered a depletion period. Thus, only data from the last 6 wk of the experiments were used in the statistical analyses. Egg production was recorded daily and feed consumption (determined as feed disappearance) was measured weekly throughout the 8-wk-long experiments. Consumption of the assay AA (mg/d) was calculated from the mean daily feed consumption (g/d) over the last 6 wk of each experiment and the dietary true digestible AA content (%). This latter content was calculated from the analyzed total AA content of the basal diet multiplied by the analyzed digestibility coefficient plus the inclusion of crystalline AA. Once a week, eggs collected over a 48-h period were weighed and the egg mass

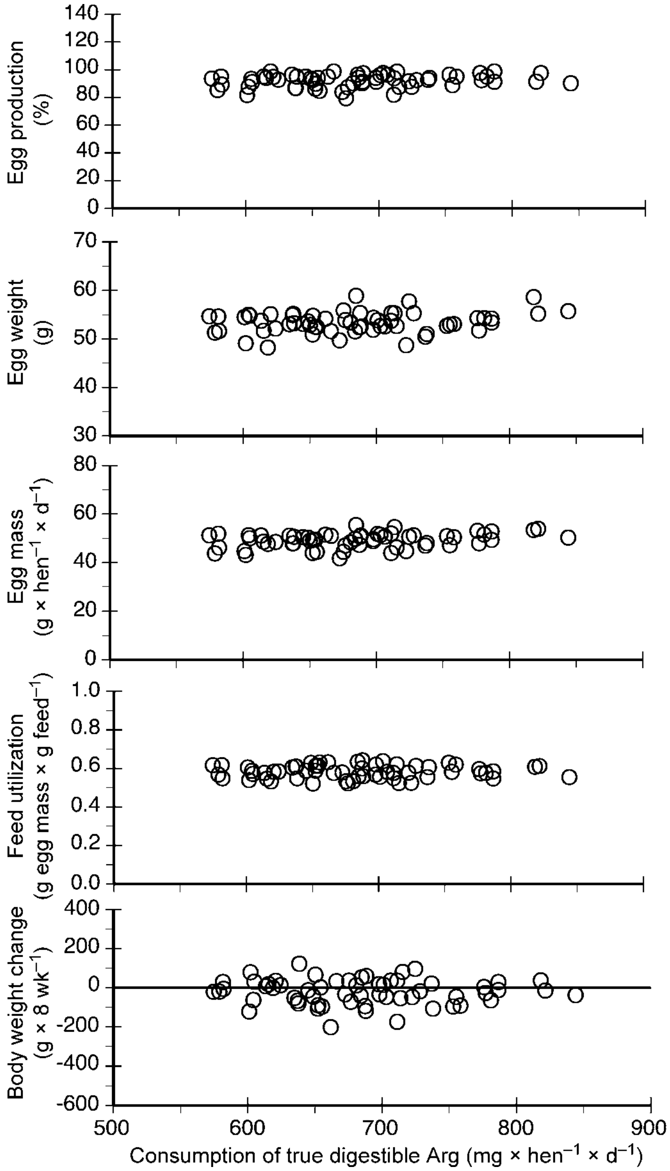

Figure 1. Responses to consumption of true digestible Arg in experiment 1 (28 to 34 wk of age). A broken-line regression could not be fitted ($P > 0.05$) to any of the responses shown. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data (n = 60) collected over 6 wk from 1 cage containing 2 hens.

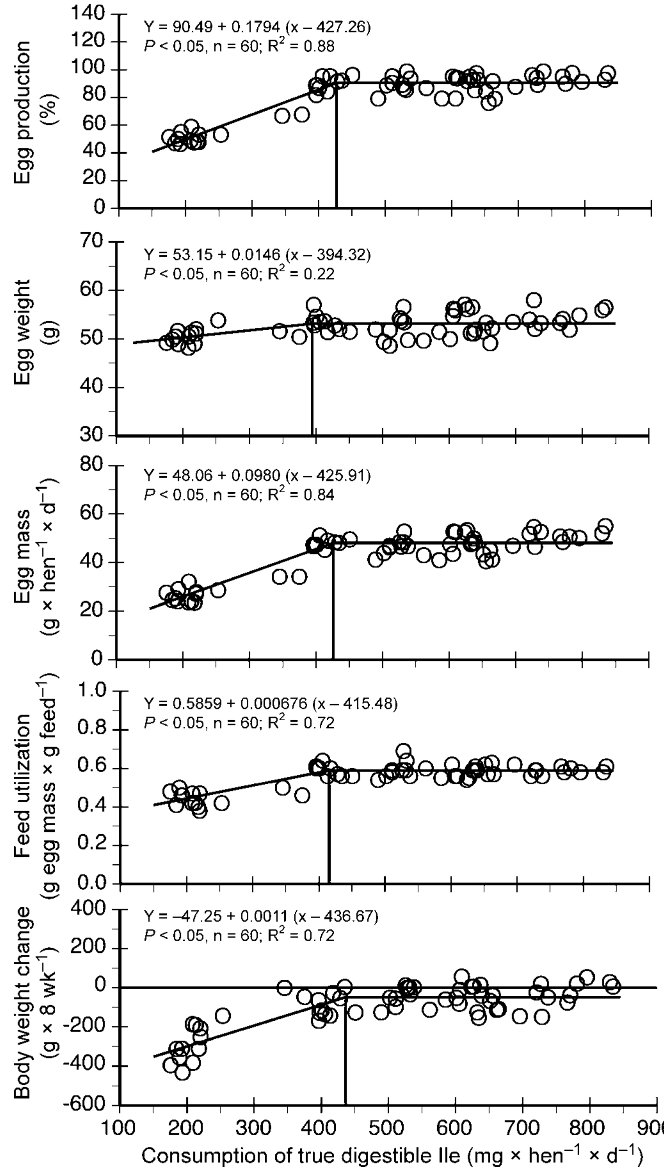

Figure 2. Responses to consumption of true digestible Ile in experiment 1 (28 to 34 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

calculated by multiplying the week's egg-production rate by the egg weight. Feed utilization was calculated as grams of egg mass divided by grams of feed consumed. Mortalities were recorded throughout the study with egg production and feed consumption rates adjusted accordingly.

Eggs collected over a 24-h period during wk 4 of Exp. 1 (i.e., hens at 30 wk of age) were saved for measurement of specific gravity using the flotation technique. Briefly, saline solutions were prepared with feed-grade NaCl and tap water to make densities from 1.065 to 1.095 g/cm^3 in increments of 0.002 g/cm^3. Eggs were placed sequentially in saline solutions, beginning with the lowest density, until the eggs floated for at least 5 s. The density at which each individual egg floated was recorded as its specific gravity. All specific gravity measurements were carried out in a temperature-controlled room set at 18°C. Eggs collected over a 24-h period during wk 5 of Exp. 1 (i.e., hens at 31 wk of age) were saved for measurement of egg contents. Eggs were weighed, broken, and separated into yolk, shell, and albumen fractions. Wet weights were recorded separately for all 3 components from each egg. The corresponding dry weights were recorded after drying in a forced-air drying oven at 70°C for 24 h. The percentage of egg solids was calculated as the sum of dried weights of yolk and albumen divided by the wet weight of the whole egg. Eggs collected over a 24-h period during wk 6 of Exp. 1 (i.e., hens at 32 wk of age) were weighed and used for determination of Haugh units. The albumen height of each egg was measured using an elec-

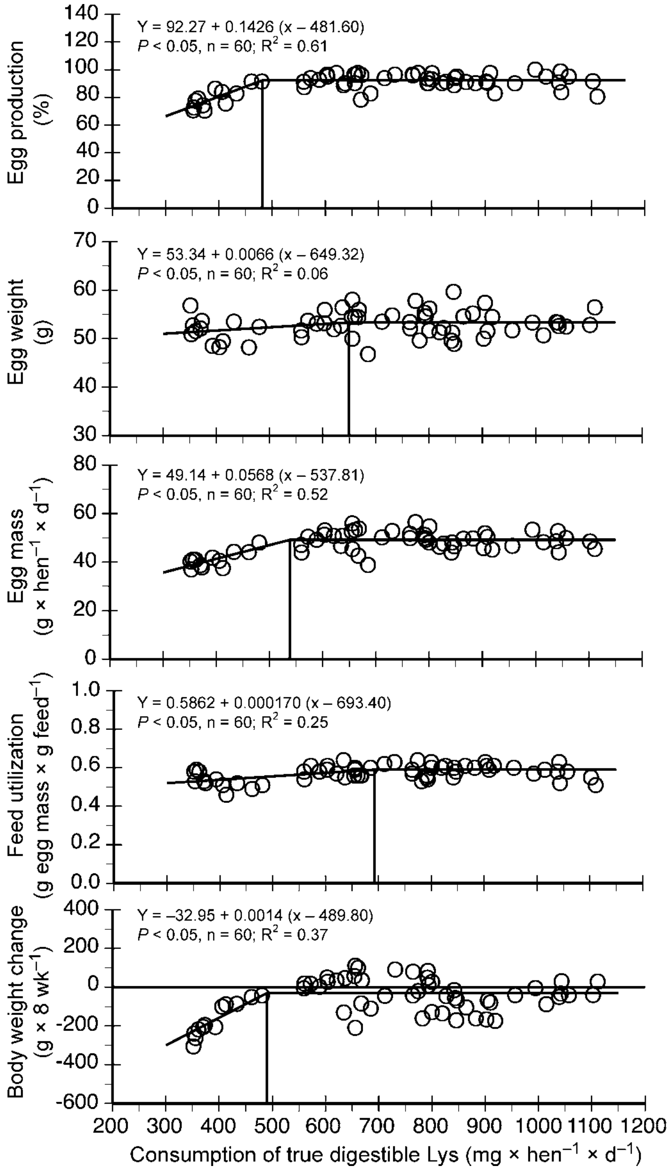

Figure 3. Responses to consumption of true digestible Lys in experiment 1 (28 to 34 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

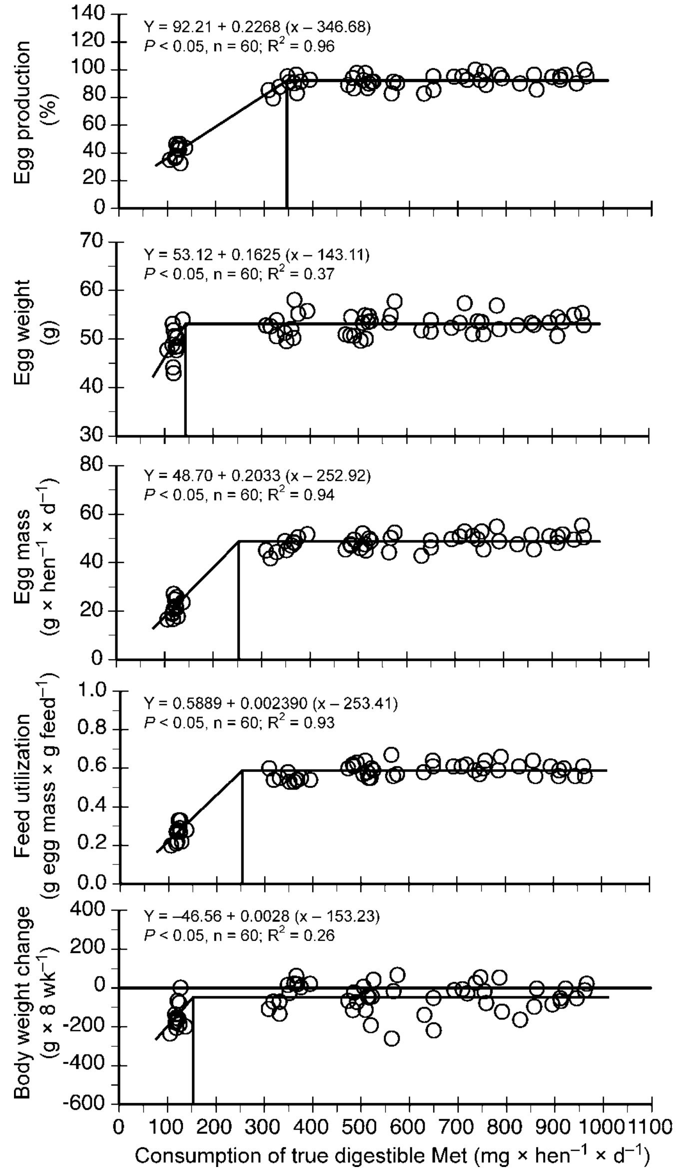

Figure 4. Responses to consumption of true digestible Met in experiment 1 (28 to 34 wk of age and associated broken-line regression). The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

tronic tripod albumen-height gauge (Technical Services and Supplies, York, UK) with the Haugh units subsequently calculated from the records of egg weight (g) and albumen height (mm) as Haugh unit = 100 × Log(albumen height − 1.7 − egg weight$^{0.37}$ + 7.57). The albumen height was measured in a temperature-controlled room set at 13°C. Hens were weighed at the beginning and end of Exp. 1 and BW change over the 8-wk-long experiment calculated.

Statistical Analyses

In each of the 7 dose-response assays, the requirement for the assayed AA was determined in a randomized complete block design with 6 dietary treatments (i.e., 5 levels of the assayed AA and 1 control diet) and 12 blocks (Morris, 1999). Thus, each block consisted of 6 cages, to which the 5 assay diets and the 1 control diet were randomly distributed. The cage location within the barn served as the blocking criterion and the experimental unit was 1 cage containing 2 hens. The requirements for digestible AA were calculated with the single-slope broken-line regression model (Robbins, 1986) using the nonlinear modeling option in JMP (version 6.0.3; SAS Institute, Cary, NC) with the consumption of the assayed AA (mg/d) as the independent variable (i.e., only data from the 5 cages receiving the amino acid assay diets were used). Block was not included in the broken-line regression model. The R^2 values were calculated as 1 − (sum of squared errors/total sum of squares), where the sum

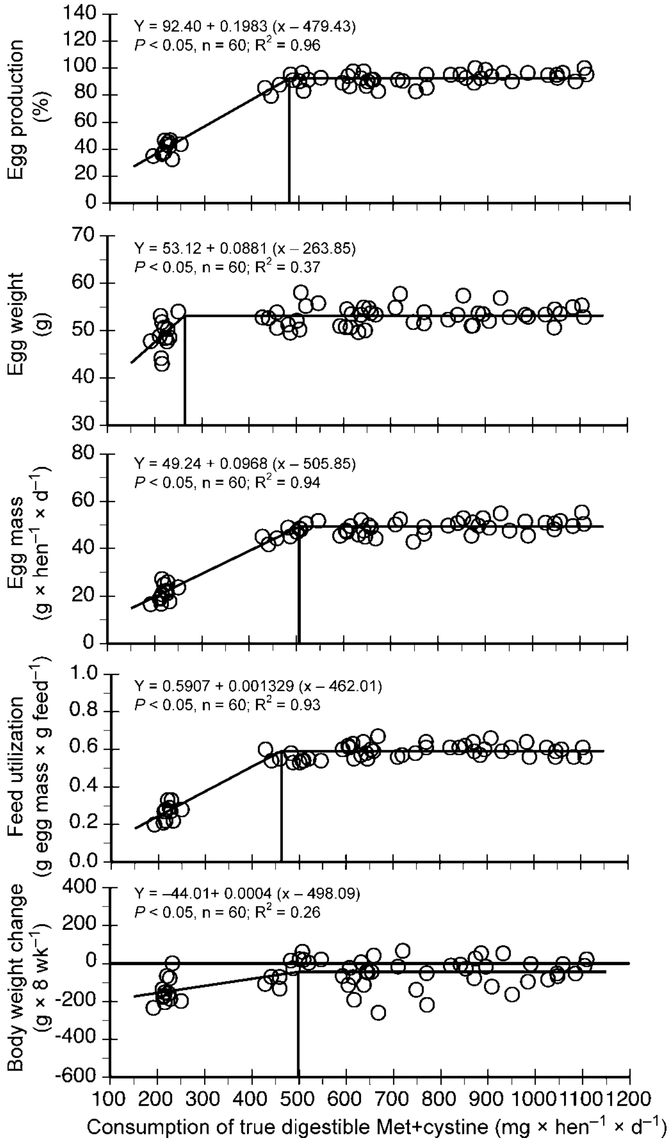

Figure 5. Responses to consumption of true digestible TSAA in experiment 1 (28 to 34 wk of age) and associated broken-line regression. Graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

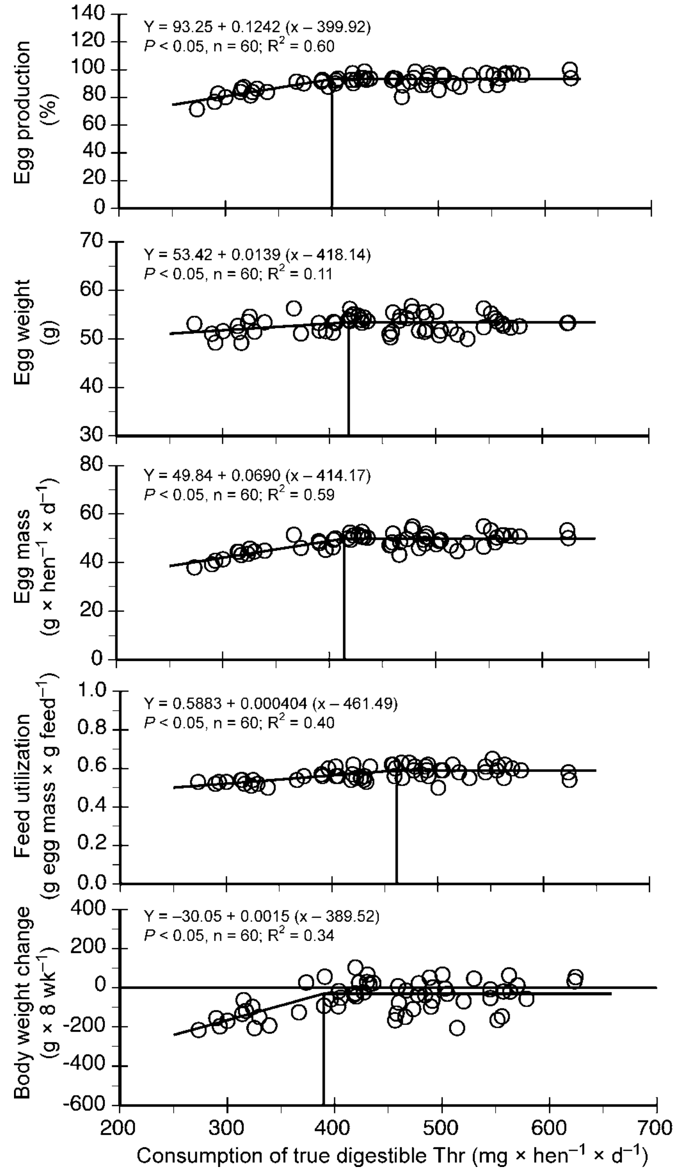

Figure 6. Responses to consumption of true digestible Thr in experiment 1 (28 to 34 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

of squared errors were derived from the broken-line regression model. Because the nonlinear modeling option in JMP only reports the sum of squared errors, the total sum of squares were calculated after fitting an overall mean to the data using the Fit-Y-by-X option in JMP. Feed consumption data were analyzed by ANOVA with block and dietary treatment as the independent variables; treatment effects were separated using linear, quadratic, and cubic orthogonal polynomial contrasts (Morris, 1999). The maximal response and associated SE (n = 60) from each of the 7 assays, determined using the broken-line model, was compared with the corresponding response from the control-fed hens (n = 12) using a 2-tailed *t*-test taking the unequal replications into account (Snedecor, 1946). If the broken-line regression model did not converge, ANOVA was performed with block and diet as the independent variables and, if the main effect of diet was not significant, the overall mean was compared with that of the control diet using a 2-tailed *t*-test. Probability values less than or equal to 0.05 were considered significant in all comparisons.

RESULTS

Exp. 1 (Hens 28 to 34 Wk of Age)

The hens were generally in good health during the experiment; 3 of the 1,008 hens (0.3%) died of reasons considered unrelated to the dietary treatments. The responses to consumption of Arg, Ile, Lys, Met, TSAA, Thr, Trp, and Val are shown in Figures 1 to 8, respectively.

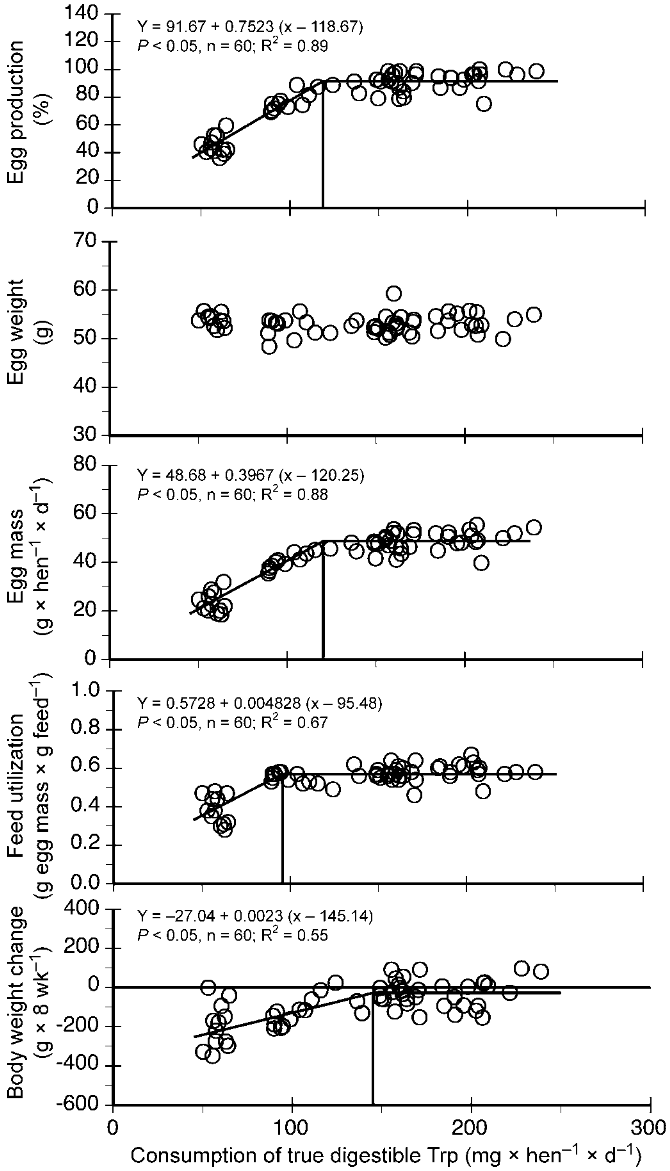

Figure 7. Responses to consumption of true digestible Trp in experiment 1 (28 to 34 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens. The broken-line regression did not converge for egg weight data ($P > 0.05$).

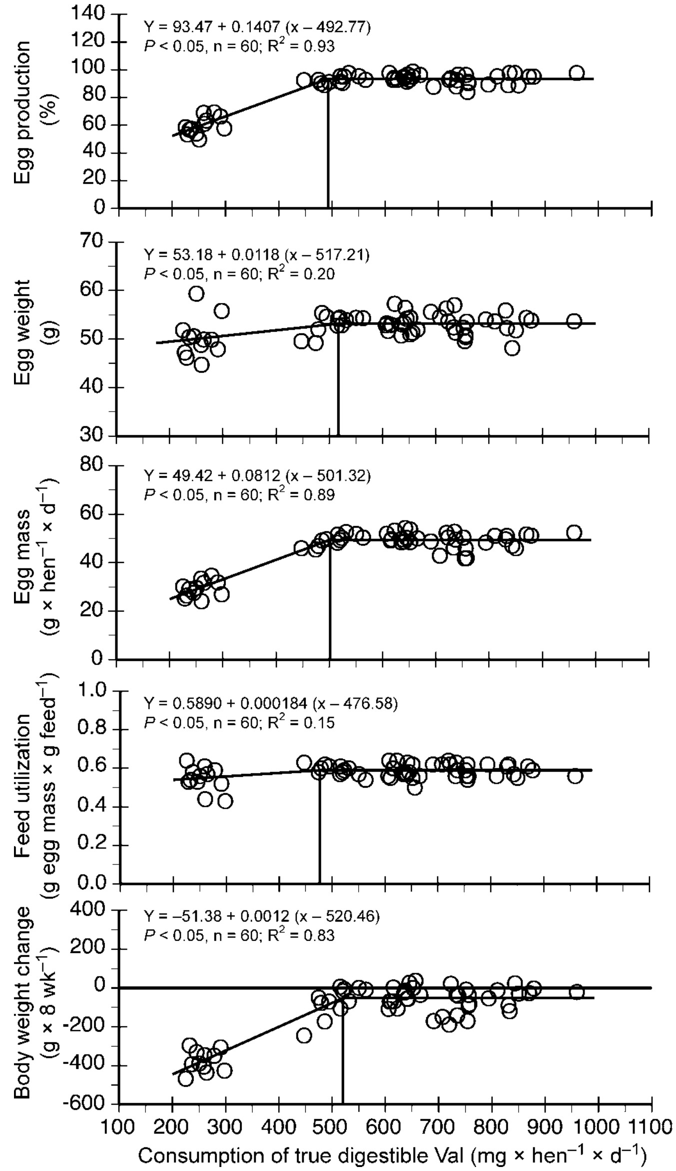

Figure 8. Responses to consumption of true digestible Val in experiment 1 (28 to 34 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

Hens fed the 5 Arg assay diets consumed between 574 and 843 mg/d of true digestible Arg, yet there were no responses to consumption of Arg. Table 5 summarizes the requirements for all the responses as determined using the broken-line regression model. Hens fed the graded levels of AA generally responded by increasing the feed consumption in a linear or curvilinear manner (Table 6). Production parameters of hens fed the AA-supplemented basal diet were inferior ($P < 0.05$) to those observed from hens fed the industry-type control diet, even when the AA diets supplied the assayed AA above its requirement (Table 7). There was no difference in feed consumption of control-fed hens among the 7 AA assays and an overall mean was calculated (84.2 ± 0.5 g/d of feed; mean ± SEM, n = 84).

Exp. 2 (Hens 52 to 58 Wk of Age)

The hens were generally in good health in the period between Exp. 1 and 2 and during Exp. 2; there was no mortality. The responses to consumption of Lys, Met, and TSAA are shown in Figures 9 to 11, respectively. Table 8 summarizes the requirements for all the responses as determined using the broken-line regression model. As observed in Exp. 1, hens in Exp. 2 responded to the AA diets by varying feed consumption in a linear or curvilinear matter (Table 9). Production parameters of hens fed the AA-supplemented basal diet were inferior ($P < 0.05$) to those observed from hens fed the industry type control diet even when the AA diets supplied the assayed AA above its requirement (Table 10). Across the 2 AA assays,

Table 5. Requirements for true digestible amino acids determined using the broken-line regression in experiment 1 (28 to 34 wk of age)[1]

Response	Lys	Ile	Met	TSAA[2]	Thr	Trp	Val
	mg × hen^{-1} × d^{-1}						
Egg production (%)	482 (23)	427 (13)	347 (11)	479 (15)	400 (16)	119 (4)	493 (14)
Egg weight (g)	649 (176)	394 (85)	143 (15)	264 (28)	418 (52)	ND	517 (91)
Egg mass (g × hen^{-1} × d^{-1})	538 (63)	426 (17)	253 (82)	506 (14)	414 (16)	120 (5)	501 (19)
Feed utilization (g of egg mass/g of feed)	693 (78)	415 (22)	253 (83)	462 (18)	461 (26)	95 (5)	477 (136)
BW change (g × 8 wk^{-1})	490 (42)	437 (24)	153 (37)	498 (97)	390 (24)	145 (17)	520 (21)
Egg solids (%)	503 (51)	ND[3]	628 (153)	745 (147)	372 (45)	123 (31)	512 (137)
Albumen content (g/100 g of egg)	478 (64)	478 (161)	ND	ND	418 (123)	161 (40)	590 (109)
Yolk content (g/100 g of egg)	483 (40)	ND	409 (200)	554 (234)	325 (31)	ND	ND
Eggshell content (g/100 g of egg)	ND	487 (85)	ND	ND	ND	556 (90)	ND

[1]Values are means and associated SE from the broken-line regression (n = 60).

[2]Graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet.

[3]ND: a broken-line regression could not be fitted and a requirement could therefore not be determined.

control-fed hens consumed 95.0 ± 1.2 g/d of feed (mean ± SEM, n = 24).

DISCUSSION

The intent with the experimental design was to have 2 of the 5 assay diets supply the assayed AA below the estimated requirement; 1 diet supply the assayed AA at the estimated requirement, and 2 diets supply the assayed AA above the estimated requirement. However, the requirements for Lys and Met were overestimated in Exp. 1, such that only 1 assay diet supplied the assayed AA below the observed requirement. In other words, only 1 of the 5 assay diets was deficient in the assayed AA, lowering the confidence in the calculated AA requirement needed to calculate the ideal AA profile. The Lys and Met requirements were therefore reevaluated in Exp. 2 with the same hens using the same methodology as in Exp. 1, albeit with lower dietary contents of the assayed AA. Consequently, the breakpoints for Lys, Met, and TSAA were better defined in Exp. 2. Despite the differences in the age of the hens between the 2 experiments, the requirements for true digestible Lys, Met, and TSAA for maximal egg mass were similar. Accordingly, the ideal Met:Lys ratios (47 and 52% in Exp. 1 and 2, respectively) and TSAA:Lys ratios (94 and 96% in Exp. 1 and 2, respectively) corresponded well between the 2 experiments, indicating that the ratios for Met and TSAA determined in Exp. 1 were acceptable. Although Baker et al. (1996) recommended that requirements for Met and Cys should be considered separately to obtain a TSAA requirement for chickens, graded levels of TSAA were created by addition of Met, not Met+Cys, in the present study. This decision was made because feed-grade crystalline Cys is not available, and increasing the dietary TSAA content in commercial laying hen diets would be done by addition of Met. Despite the additions of Met only, the observed Met:Cys ratio (calculated from the observed true digestible Met and TSAA requirements of 253 and 506 mg/d, respectively) was 50%, similar to the Met:Cys ratio reported as being optimal for growing broiler chickens and pigs (NRC, 1994; Baker et al., 1996; NRC, 1998; Lewis, 2003).

AA Requirements

The AA requirements used to calculate the ideal AA profile were determined with the broken-line regression model. This method is considered the best for obtaining the ideal ratios among AA, whereas curvilinear models

Table 6. Feed consumption of hens fed the amino acid-supplemented basal diet in Exp. 1 (28 to 34 wk of age)[1]

Amino acid assayed	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM[2]
	g × hen^{-1} × d^{-1}					
Lys[3,4]	77.8	89.0	84.6	80.5	81.4	1.7
Arg[5]	89.0	87.9	82.1	83.1	80.6	1.6
Ile[3,4,5]	55.7	79.8	85.2	80.9	79.7	1.9
Met[3,4,5]	62.7	86.3	81.6	81.5	82.7	1.8
Thr[3,4]	82.6	90.5	85.2	82.8	82.2	1.8
Trp[3,5]	61.6	74.7	87.9	80.9	82.5	2.0
Val[3,4,5]	53.8	83.3	85.1	86.7	81.8	1.3

[1]The data for Met and TSAA were from the same data set; feed consumption for Met and TSAA were therefore equal.

[2]Pooled standard error of the mean (n = 12).

[3]Quadratic effect ($P < 0.05$).

[4]Cubic effect ($P < 0.05$).

[5]Linear effect ($P < 0.05$).

Table 7. Comparison of responses of hens fed amino acid-adequate assay diets with the control-fed hens in Exp. 1 (28 to 34 wk of age)[1]

Response and assayed amino acid	Control	Maximal response[2]	*P*-value of difference[3]
Egg production (%)			
Lys	97.3 (0.7)	92.3 (0.7)	<0.01
Arg	96.7 (0.9)	92.2 (0.6)	<0.01
Ile	96.1 (0.8)	90.5 (1.0)	<0.05
Met	97.4 (0.8)	92.2 (0.7)	<0.01
TSAA[4]	97.4 (0.8)	92.4 (0.7)	<0.01
Thr	97.0 (0.9)	93.3 (0.6)	<0.01
Trp	96.4 (0.5)	91.7 (1.1)	NS
Val	95.4 (1.0)	93.5 (0.6)	NS
Egg weight (g)			
Lys	56.0 (0.4)	53.3 (0.4)	<0.01
Arg	56.8 (0.3)	53.4 (0.3)	<0.001
Ile	56.4 (0.7)	53.2 (0.3)	<0.001
Met	57.2 (0.7)	53.1 (0.3)	<0.001
TSAA[4]	57.2 (0.7)	53.1 (0.3)	<0.001
Thr	57.5 (0.6)	53.4 (0.3)	<0.001
Trp	57.0 (0.4)	53.1 (0.2)	<0.001
Val	57.0 (0.7)	53.2 (0.4)	<0.001
Egg mass (g × hen^{-1} × d^{-1})			
Lys	54.5 (0.6)	49.1 (0.5)	<0.001
Arg	54.4 (0.9)	49.2 (0.4)	<0.001
Ile	54.2 (0.8)	48.1 (0.6)	<0.001
Met	55.7 (0.7)	48.7 (0.5)	<0.001
TSAA[4]	55.7 (0.7)	49.2 (0.5)	<0.001
Thr	55.8 (0.7)	49.8 (0.4)	<0.001
Trp	54.9 (0.5)	48.7 (0.6)	<0.001
Val	54.3 (1.0)	49.4 (0.5)	<0.001
Feed utilization (g of egg mass × kg feed^{-1})			
Lys	655 (10)	586 (6)	<0.001
Arg	647 (10)	583 (4)	<0.001
Ile	638 (6)	586 (6)	<0.001
Met	649 (10)	589 (7)	<0.001
TSAA[4]	649 (10)	591 (6)	<0.001
Thr	663 (7)	588 (6)	<0.001
Trp	664 (8)	573 (8)	<0.001
Val	633 (8)	589 (6)	<0.01
Body weight change (g × hen^{-1} × 8 wk^{-1})			
Lys	30 (9)	−33 (11)	<0.05
Arg	45 (15)	−20 (65)	NS
Ile	42 (11)	−47 (11)	<0.001
Met	44 (12)	−47 (11)	<0.001
TSAA[3]	44 (12)	−44 (11)	<0.001
Thr	27 (11)	−30 (10)	<0.05
Trp	39 (13)	−27 (12)	<0.05
Val	54 (11)	−51 (9)	<0.001

[1]The values are means (SEM), n = 12 for control and n = 60 for assay diets.

[2]Response at the amino acid requirement, determined using the single-slope broken-line model.

[3]Two-tailed *t*-test; NS, not significant ($P > 0.05$).

[4]Graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet.

such as exponential or quadratic curve fitting are better suited to establish the AA requirements for optimal performance (Mack et al., 1999; Baker et al., 2002; Robbins et al., 2006). Typically, the broken-line regression method results in lower AA requirements than when a nonlinear curve fitting is applied to the same data set (Fisher et al., 1973). However, the broken-line regression model has the advantages of a clearly defined breakpoint (i.e., the requirement) at a dietary AA consumption that marginally limits performance, both necessary to determine the ideal AA profile (Mack et al., 1999). Although the absolute AA requirements (mg/d) are reported herein, they are only valid for the particular hens in the particular experimental settings in the present study and should not necessarily be used in commercial settings, in part because they were determined using the broken-line method and not a curvilinear model (Mack et al., 1999; Baker et al., 2002), and in part because egg production of control-fed hens was superior to that of hens fed the AA assay diets.

Among the AA requirements, Lys is especially important, because it is used as the basis for setting the requirements for all other AA in the ideal AA profile (Baker et al., 2002). In Exp. 1, the true digestible Lys requirement, determined using the broken-line regression model, for maximal egg mass and feed utilization was 538 and 693 mg/d, respectively, similar to the requirements (540 and 720 mg/d apparent digestible Lys, respectively) reported by Schutte and Smink (1998) in 24-

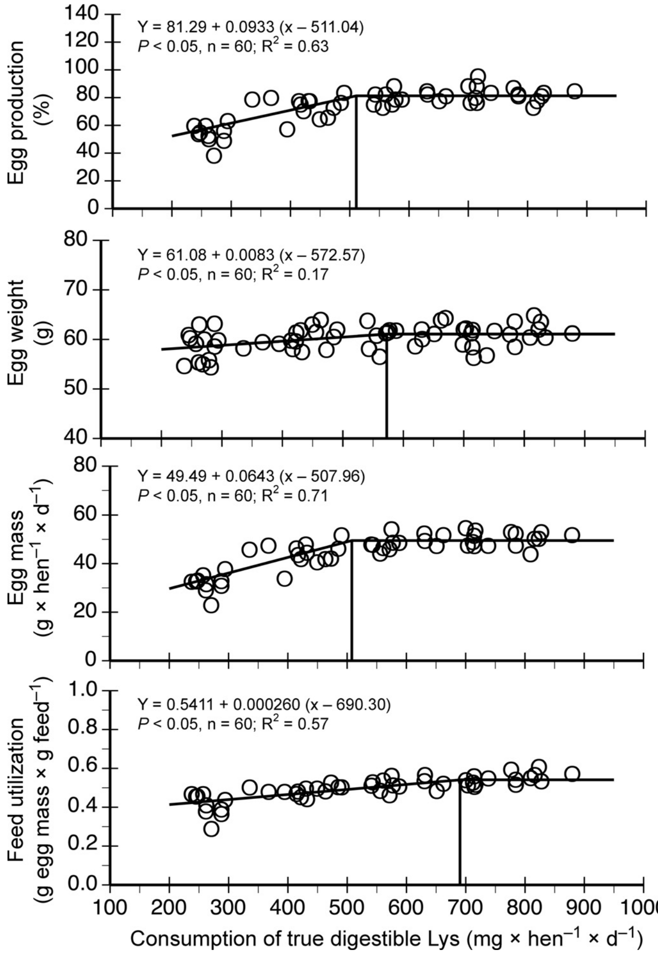

Figure 9. Responses to consumption of true digestible Lys in experiment 2 (52 to 58 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

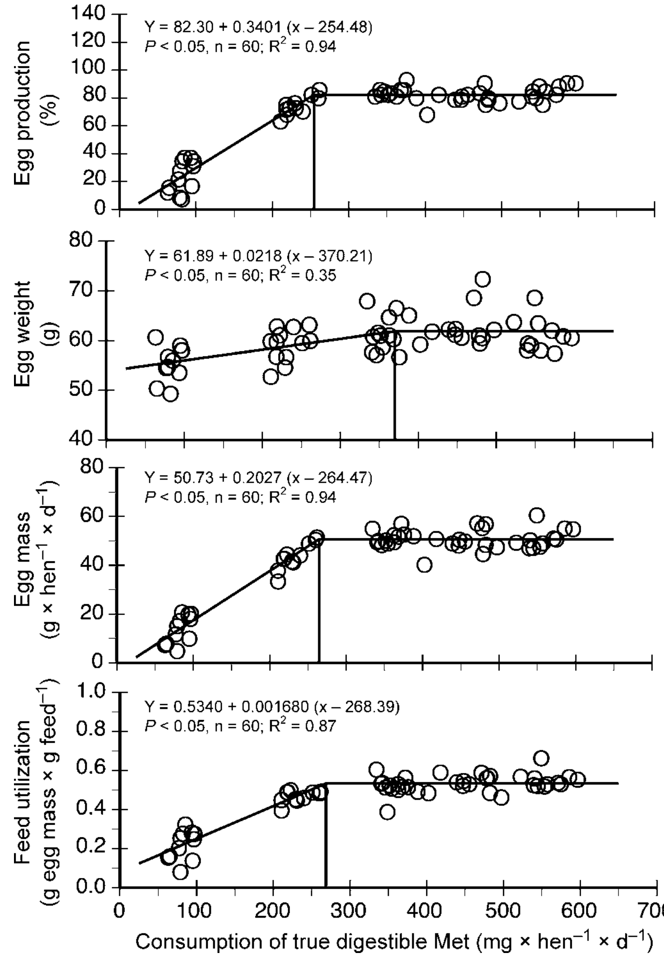

Figure 10. Responses to consumption of true digestible Met in experiment 2 (52 to 58 wk of age) and associated broken-line regression. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

to 36-wk-old white Leghorn-type hens (Lohmann LSL) using an exponential model and similar to the 700 mg/d of digestible Lys recommended by the CVB (1996) for optimal feed conversion. The NRC (1994) recommends a total Lys consumption of 690 mg/d to maximize egg yield (egg mass), corresponding to 593 mg/d of true digestible Lys when applying a mean true Lys digestibility of 86% in corn and soybean meal (NRC, 1994). Coon and Zhang (1999) reported digestible Lys requirements of 705 and 636 mg/d for 33- to 49-wk-old and 35- to 47-wk-old laying hens, respectively, to maximize egg mass of white Leghorn-type hens (Hy-Line W-36). In general, the Lys requirement determined in the present study corresponded well with those reported in literature.

Consumption of Arg did not elicit any responses in Exp. 1, indicating that the basal diet supplied sufficient amounts of Arg to meet the requirement of the hens for the responses measured or that Arg was not the first-limiting AA in the assay diets. Coon and Zhang (1999) demonstrated a digestible Arg requirement of 968 and 791 mg/d for 33- to 49-wk-old and 35- to 47-wk-old laying hens, respectively, whereas the NRC (1994) suggests a requirement for total Arg of 700 mg/d based on a model by Hurwitz and Bornstein (1973). The latter value corresponds to 637 mg/d of true digestible Arg when applying a mean true AA digestibility of 91% in corn and soybean meal (NRC, 1994). Jais et al. (1995) suggested an ideal Arg:Lys ratio of 82% for laying hens, corresponding to a true digestible Arg requirement of 441 mg/d for the hens in Exp. 1 (calculated as 82% of the observed Lys requirement of 538 mg/d). In Exp. 1 of the present study, the basal diet provided 0.69% true digestible Arg, resulting in a mean true digestible Arg consumption of 668 mg/d by hens fed the Arg-1 diet, which was lower than that recommended by Coon and Zhang (1999), but greater than that recommended by the NRC (1994) and Jais et al. (1995). If the NRC (1994) requirement of 637 mg/d of true digestible Arg is accepted, then not only was the Arg requirement met by the Arg-1 diet, but Phe became more limiting than Arg in the Arg-1 diet when compared with the NRC (1994) Phe requirement. However, Phe was not limiting in the Arg assay diets if the Arg and Phe requirements suggested by Jais et al. (1995) or Coon and Zhang (1999) are accepted. Yet another potential cause

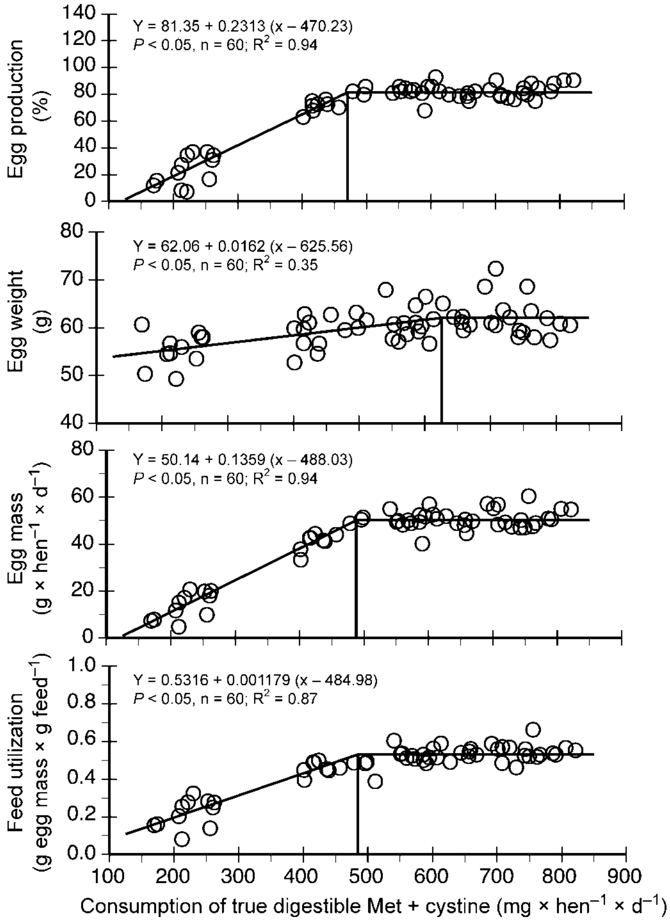

Figure 11. Responses to consumption of true digestible TSAA in experiment 2 (52 to 58 wk of age) and associated broken-line regression. Graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet. The experimental diets were fed for 8 wk with the first 2 wk considered a depletion period. Each dot (○) represents data collected over 6 wk from 1 cage containing 2 hens.

for the lack of response to Arg is a Lys-Arg antagonism, in which high dietary levels of Lys decrease the Arg utilization (Jones, 1964; Austic and Scott, 1975) and, as a result, reduce feed utilization (Mendes et al., 1997; Brake et al., 1998; Kidd and Kerr, 1998) and egg production. However, all the diets were designed with incrementally increasing contents of the nonassayed crystalline AA to avoid AA antagonisms and imbalances. Hence, the Lys content was similar in the Arg-2 and Arg-3 diets (and in the Arg-4 and Arg-5 diets), and, because the Arg content increased in all Arg assay diets, the Arg:Lys ratio (calculated from analyzed values; data not shown) therefore increased from 74 to 78% in the Arg-2 and Arg-3 diets, respectively (and from 71 to 75% in the Arg-4 and Arg-5 diets, respectively). Despite the increases in Arg:Lys ratios in the 2 pairs of Arg diets, feed utilization or egg production did not differ, indicating that a Lys-Arg antagonism did not occur in the present experiment.

The observed requirements for true digestible Met and TSAA to maximize egg mass (253 and 506 mg/d, respectively) were similar to those recommended by the NRC (1994), when taking digestibility into account. However, results of dose-response assays by Schutte et al. (1994), Waldroup and Hellwig (1995), and Coon and Zhang (1999) indicate that the Met and TSAA requirements for laying hens are well above the NRC (1994) levels.

The requirement for true digestible Thr to maximize egg mass observed in Exp. 1 of the present study (414 mg/d) agreed well with the 453 and 462 mg/d of total Thr reported by Ishibashi et al. (1998) and Faria et al. (2002) for white Leghorn-type laying hens and with the 430 mg/d of digestible Thr reported by Coon and Zhang in their Exp. 3 with 35- to 47-wk-old Hy-Line W-36 hens. However, in their Exp. 2, Coon and Zhang (1999) found the digestible Thr requirement to be 560 mg/d in 33- to 49-wk-old Hyline-W-36 hens, considerably greater than in the present study.

The observed requirement for true digestible Trp (120 mg/d) in the present study was similar to the 122 mg/d of digestible Trp reported by Coon and Zhang (1999) for 33- to 49-wk-old laying hens and the 149 mg/d of total Trp reported by Harms and Russell (2000b) for 30- to 36-wk-old hens. However, the NRC (1994) recommends 160 mg/d of total Trp, greater than that observed in the present study.

The Ile requirements reported by the NRC (1994) (650 mg/d of total Ile), Harms and Russell (2000a) (601 mg/d of total Ile), and Coon and Zhang (1999) (555 to 603 mg/d of digestible Ile, depending on age) are greater than that observed in the present study (426 mg/d of true digestible Ile). Yet, the Ile requirement from the present study was comparable to the 469 mg/d of total Ile reported by Shivazad et al. (2002) for 37- to 43-wk-old laying hens when applying a mean true Ile digestibility of 91% in corn and soybean meal (NRC, 1994).

Reported Val requirements for white Leghorn-type laying hens are greater than those observed in the present study (501 mg/d of true digestible Val). The NRC (1994)

Table 8. Requirements for true digestible amino acids determined using the broken-line regression in experiment 2 (52 to 58 wk of age)[1]

Response	Lys	Met	TSAA[2]
	mg × hen^{-1} × d^{-1}		
Egg production (%)	511 (34)	254 (8)	470 (15)
Egg weight (g)	573 (110)	370 (56)	626 (75)
Egg mass (g × hen^{-1} × d^{-1})	508 (27)	264 (8)	488 (16)
Feed utilization (g of egg mass/g of feed)	690 (59)	268 (12)	485 (18)

[1]Values are means and associated SE from the broken-line regression (n = 60).

[2]Graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet.

Table 9. Feed consumption of hens (g × hen^{-1} × d^{-1}) fed the amino acid-supplemented basal diet in experiment 2 (52 to 58 wk of age)[1]

Amino acid	Diet 1	2	3	4	5	SEM[2]
Lys[3,4]	78.2	91.9	94.2	97.3	90.2	1.9
Met[3,4,5]	60.8	92.3	97.5	92.3	91.5	2.1

[1]The data for Met and TSAA were from the same data set; feed consumption for Met and TSAA were therefore equal.

[2]Pooled standard error of the mean (n = 12).

[3]Linear effect ($P < 0.05$).

[4]Quadratic effect ($P < 0.05$).

[5]Cubic effect ($P < 0.05$).

recommends 700 mg/d of total Val, whereas Coon and Zhang (1999) reported that laying hens needed between 646 and 731 mg/d of digestible Val. Harms and Russell (2001) reported a total Val requirement of 619 mg/d for 41- to 47-wk-old Hy-Line W-36 hens.

Egg Production

The observed mean egg production, egg weight, and egg mass of control-fed hens were comparable to those listed in the 2006–2008 W-36 Commercial Management Guide (i.e., 93.6%, 57.8 g, and 54.1 g/d, respectively for 28- to 34-wk-old hens; and 83%, 62.5 g, and 51.9 g/d, respectively for 52- to 58-wk-old hens). However, the observed egg production parameters from hens fed the AA assay diets were generally inferior to those observed for the control-fed hens, even when the AA requirements were met. Although such observations were discussed by Baker et al. (2002) for broiler chickens, other studies of AA requirements for laying hens mentioned previously have not included a positive control diet enabling direct comparisons. The difference in performance between hens fed the control diet and that of hens consuming adequate AA from the assay diets in the present study cannot readily be explained by a difference in feed or (calculated) ME consumption because the consumption of feed and ME was similar between the 2 groups of hens. A careful comparison of the analyzed dietary AA contents with that of the requirements revealed that the assayed AA were first limiting in their respective assay diets (except perhaps Arg, as discussed previously).

The AA-supplemented basal diet was similar in concept to low-CP diets, in which soybean meal is partially replaced by a mixture of corn and crystalline AA, resulting in a relatively low CP content. The N contributed by true digestible essential AA supplied approximately 44% of the total N in the assay diets when the AA requirements were met (data not shown), indicating an essential:nonessential AA ratio of approximately 44%. In broiler chickens and pigs, the optimal essential:nonessential AA ratio is approximately 50% (Deschepper and Groote, 1995; Heger et al., 1998), suggesting that the assay diets supplied sufficiently nonspecific N, but that energy and Gly may have had to be expended excreting the

Table 10. Comparison of egg-production responses of hens fed amino acid-adequate assay diets with the control-fed hens in experiment 2 (52 to 58 wk of age)[1]

Response and assayed amino acid	Control	Maximal response[2]	*P*-value of difference[3]
Egg production (%)			
Lys	85.7 (1.1)	81.0 (0.9)	<0.05
Met	86.9 (1.8)	82.3 (1.2)	<0.05
TSAA[4]	86.9 (1.8)	82.3 (2.2)	<0.05
Egg weight (g)			
Lys	63.7 (0.7)	61.1 (0.5)	<0.05
Met	64.3 (0.7)	61.7 (0.6)	<0.05
TSAA[4]	64.3 (0.7)	62.3 (0.8)	NS
Egg mass (g × hen^{-1} × d^{-1})			
Lys	54.6 (1.0)	49.4 (0.5)	<0.001
Met	55.8 (1.1)	50.7 (0.7)	<0.01
TSAA[4]	55.8 (1.1)	50.7 (1.2)	<0.05
Feed utilization (g of egg mass × kg feed^{-1})			
Lys	582 (8)	537 (9)	<0.05
Met	583 (12)	532 (10)	<0.05
TSAA[4]	583 (12)	534 (8)	<0.05

[1]The values are means (SEM), n = 12 for control and n = 60 for assay diets.

[2]Response at the amino acid requirement, determined using the single-slope broken-line model.

[3]Two-tailed *t*-test; NS, not significant ($P > 0.05$).

[4]Graded levels of TSAA were created by addition of Met (not Met+Cys) to the basal diet.

Table 11. Ideal amino acid profiles[1] for laying hens determined in the present study (28 to 34 wk of age) and calculated from reported amino acid requirements

Amino acid	Present study[2]	NRC (1994)[3]	Jais et al. (1995)[4]	CVB (1996)[5]	Coon and Zhang (1999)[5]	Leeson and Summers (2005)[6]
Lys	100	100	100	100	100	100
Arg	—[7]	101	82	—	130	103
Ile	79	94	76	79	86	79
Met	47	43	44	50	49	51
TSAA	94	84	—	93	81	88
Thr	77	68	76	66	73	80
Trp	22	23	16	19	20	21
Val	93	101	64	86	102	89

[1]Lysine requirement set at 100%.

[2]Based on true digestible requirements for maximal egg mass in experiment 1 (Table 5).

[3]Based on total amino acid requirements.

[4]Based on N balance.

[5]Based on digestible amino acid requirements.

[6]Based on total amino acid requirements for 32- to 45-wk-old laying hens.

[7]The Arg:Lys ratio is estimated to be 107 or less; see text.

"excess" nonessential AA and giving a possible explanation for the relatively low production of hens fed the assay diets. Dean et al. (2006) demonstrated that low-CP diets for broilers were deficient in Gly+Ser, even when the NRC-recommended dietary levels were met. The NRC (1994) does not list a minimum recommended dietary content of Gly+Ser for laying hens, but recommends between 0.47 and 0.70% total Gly+Ser for immature Leghorn-type chickens depending on their age. However, the basal diet supplied 1.30% total Gly+Ser in Exp. 1, and consumption of these AA were likely not limiting egg production.

The analyzed content of Phe in the basal diet (0.47%) was similar to that recommended by the NRC (1994). However, because the feed consumption was lower in the present study than that estimated by the NRC (1994), the calculated consumption of true digestible Phe was less than that recommended by the NRC (1994). Nevertheless, Phe was not first-limiting when compared with the assayed AA (except perhaps for Arg as discussed previously). Hence, the difference in performance between hens fed the control and assay diets cannot readily be explained by an AA or nonspecific-N deficiency.

Ideal AA Profile

In contrast to the present study, previous estimates of the ideal AA profile for laying hens were based on experiments with different ages and genetic lines of hens, conducted at different times in different environments. Baker (2003) argued that the same basal diet, the same genetic line, and the same assay period should be used in all assays of AA requirements to ensure a valid measurement of the ideal AA profile. In addition, the ideal AA profile should preferably be defined separately for maintenance and production (NRC, 1998), because the relative AA needs partitioned among these are likely to change as the hen matures. However, in this study, the AA profile for maintenance, BW growth, and egg production were combined into one profile for 28- to 34-wk-old hens (Table 11). The ideal AA profile determined in this study indicated that hens need less true digestible Met, Ile, and Val and more true digestible Thr in relation to Lys than that suggested by Coon and Zhang (1999) and that calculated from requirements published by the NRC (1994). However, the ideal Ile:Lys ratio observed in the present study corresponded well with that calculated from AA recommendations by the CVB (1996). The determined ideal AA profile agrees well with the profile calculated from AA recommendations suggested by Leeson and Summers (2005) for 32- to 45-wk-old hens and is similar to that reported by Jais et al. (1995) with the exception of Trp and Val. The ideal Met:Lys and TSAA:Lys ratios in the present study were higher than those reported by the NRC (1994) and the TSAA:Lys ratio of 75% reported by Liu et al. (2005), but agree well with the ratios suggested by the CVB (1996) and by Leeson and Summers (2005) for 32- to 45-wk-old hens. If the lowest Arg consumption observed in Exp. 1 (574 mg/d) is accepted as meeting or exceeding the requirement of the hen for Arg, the ideal Arg:Lys ratio was no greater than 107%, similar to that calculated from the Arg and Lys recommendations by NRC (1994) and CVB (1996), and less than the 130% recommended by Coon and Zhang (1999) (Table 11).

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