

Essential amino acid recommendations for Isa Brown layers during peak and post peak production

Shemil P. Macelline,^{*,†} Mehdi Toghyani,^{*,†} Peter V. Chrystal,^{*,†} Juliano C. de Paula Dorigam,[‡]
Shiva Greenhalgh,^{*,†} Peter H Selle,^{†,§} and Sonia Y. Liu ^{*,†,1}

^{*}*School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camperdown 2006 NSW, Australia;* [†]*Poultry Research Foundation, The University of Sydney, Camden 2570 NSW, Australia;* [‡]*Evonik Operations GmbH, Hanau-Wolfgang 63457, Germany;* and [§]*Sydney School of Veterinary Science, The University of Sydney, Camden 2570 NSW, Australia*

ABSTRACT The present study was designed to re-evaluate the ideal amino acid ratios of total sulphur amino acids (TSAA), Thr, Val, Ile, Trp, and Arg relative to Lys during peak and post-peak production phases in laying hens by using seven independent amino acid assays in similar experimental setting. A total of 348 twenty wk old Isa Brown laying hens were allocated to individual battery cages. Each dietary treatment included 6 replicates with 2 single cages (2 birds) as one replicate. All diets were formulated based on maize, soybean meal, and canola meal to have identical crude protein (120 g/kg) concentrations and energy density (11.9 MJ/kg) but with 5 levels of dietary concentrations of tested amino acids. Hens were offered experimental diets from 27 to 33 wk of age in experiment 1 (Exp. 1) and from 42 to 48 wk of age in experiment 2 (Exp. 2). Daily egg production and weekly egg weights were recorded,

and feed intakes were calculated for each experimental period to determine egg production rate, egg mass, and feed conversion ratio (FCR). Linear and quadratic broken line models were used to estimate amino acid requirements on egg production rate, egg mass and FCR. Overall, quadratic broken line models estimated higher amino acid requirements for egg mass, egg production rate and FCR than linear broken line models by 23, 25, and 20%, respectively. The predicted daily Lys intake recommendation was 720 mg/bird/day with linear broken line model and 897 mg/bird/day with quadratic broken line model and the recommended ideal amino acid ratios relative to Lys are 85 for TSAA, 69 for Thr, 83 for Val, 87 for Ile, 22 for Trp, and 82 for Arg based on linear broken line model and 87 for TSAA, 67 for Thr, 83 for Val, 86 for Ile, 22 for Trp, and 78 for Arg based on quadratic broken line model estimations.

Key words: amino acids, ideal amino acid ratio, laying hens, broken line models

2022 Poultry Science 101:102171

<https://doi.org/10.1016/j.psj.2022.102171>

INTRODUCTION

Global egg production increased by 50.1% (51.1 vs. 76.7 million tonnes) from 2000 to 2018 and the annual global per capita consumption was 161 eggs in 2018 (www.internationalegg.com). It is likely the global egg production will continue to grow. To meet this increasing demand, the performance of laying hens has been improved by genetic selection breeding programs coupled with better nutritional strategies. Amino acid requirements for laying hens were published in NRC (1994) and a number of relevant investigations have

been subsequently reported (Schutte et al., 1994; Ishibashi et al., 1998; Coon and Zhang, 1999; Harms and Russell, 2001; Faria et al., 2003; Leeson and Summers, 2005; Bregendahl et al., 2008; Lemme, 2009; Soares et al., 2019). However, amino acid requirements for brown layers have not been updated for nearly a decade and it is important to redetermine requirements for optimal production efficiency, welfare, and health, especially during the peak production period.

The determination of the Lys requirement is crucial, as Lys serves as the reference amino acid for the ideal amino acid ratio concept. Schutte and Smink (1998) estimated the daily digestible Lys requirement was 720 mg/bird/day to obtain the optimal feed conversion ratio (FCR) in laying hens. Two decades later, a higher estimation of 886 mg/bird daily Lys intake was reported by Spangler et al. (2019). Breed, age, performance parameters, and statistical methods may vary experimental outcomes for amino acid requirements in laying hens and

© 2022 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received May 18, 2022.

Accepted August 27, 2022.

¹Corresponding author: sonia.liu@sydney.edu.au

Table 1. Dietary composition of basal and standard diets in Experiment 1 (27 to 33 wk of age) and Experiment 2 (42 to 48 wk of age).

Item (g/kg)	Experiment 1		Experiment 2	
	Basal diet	Standard diet	Basal diet	Standard diet
Maize	696	696	710	710
Maize starch	-	-	15.0	15.0
Soybean meal	30.7	30.7	34.0	34.0
Canola meal	65.0	65.0	65.0	65.0
Soybean oil	14.0	14.0	10.0	10.0
<i>l</i> -lysine HCl	-	6.22	-	5.05
<i>d</i> -methionine	-	3.93	-	3.77
<i>l</i> -threonine	-	2.81	-	2.48
<i>l</i> -tryptophan	-	0.97	-	0.80
<i>l</i> -valine	-	3.29	-	3.12
<i>l</i> -arginine	-	3.84	-	4.11
<i>l</i> -isoleucine	-	3.26	-	3.26
<i>l</i> -leucine	1.31	1.31	2.83	2.83
<i>l</i> -histidine	-	-	0.08	0.08
Glycine	2.83	2.83	2.60	2.60
<i>l</i> -serine	3.37	3.37	3.19	3.19
Sodium bicarbonate	6.53	6.53	5.41	5.41
Potassium carbonate	6.24	6.24	6.33	6.33
Limestone flour	49.9	49.9	48.4	48.4
Limestone grit	50.0	50.0	50.0	50.0
Mono-dicalcium phosphate	17.4	17.4	8.48	8.48
Choline chloride 60%	0.40	0.40	0.40	0.40
Celites	20.0	20.0	-	-
Sand	34.3	10.0	36.4	13.8
Vitamin/mineral premix	2.00	2.00	2.00	2.00

Vitamin-mineral composition (per kg of air-dry diet): Vitamins: A 12,000 IU, D 33,000 IU, E 15 mg, K 2 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 2 mg, calcium pantothenate 0.03 mg, folic acid 0.2 mg, niacin 45 mg, biotin 0.15 μ g. Minerals: calcium 0.5%, Co 0.5 mg (as cobalt sulphate), Cu 10 mg (as copper sulphate), iodine 0.9 mg (as potassium iodine), iron 80 mg (as ferrous sulphate), Mn 80 mg (as manganous oxide), Se 0.2 mg (as sodium selenite), Zn 80 mg (as zinc oxide).

there are inconsistencies in amino acid recommendations throughout the literature (Macelline et al., 2021). The common approach is to estimate the requirement of one amino acid at one time with the exception of Bregendahl et al. (2008). However, studies on ideal amino acid ratios for laying hens with similar experimental design have not been advanced since the Bregendahl et al. (2008) study and evaluating requirements of multiple amino acids simultaneously minimizes the experimental error introduced by facility, methodology and quality of birds. Therefore, the present study was designed to estimate the requirements of Lys, TSAA, Thr, Trp, Ile, Val, and Arg for Isa Brown laying hens during peak and post-peak production periods from 27 to 33 and 42 to 48 weeks of age by using 7 sets of amino acid levels in one single feeding study.

MATERIALS AND METHODS

All experimental procedures were approved by the Research Integrity and Ethics Administration of the University of Sydney (2019/1518).

Experimental Design and Diet Formulation

Two experiments were carried out during the early and late peak production periods to estimate the requirements of Lys, TSAA, Thr, Trp, Ile, Val, and Arg in Isa Brown laying hens. There were five dietary titration levels for each amino acid. One standard diet was formulated to breeder recommendation to represent 100% amino acid

requirement (Tables 1 and 2). This standard diet was used for all seven amino acid arrays. Additional four titration concentrations were also included for each amino acid to represent 0, 33, 67, and 133% of standard requirements (Table 3). A basal diet was formulated without supplementing all tested amino acids as shown in Table 1. Then for each of the seven amino acids, the basal diet was supplemented with all amino acids but the target amino acid to form the diet representing 0% requirement. Hence, there were 4 diets for each of the 7 test amino acids plus

Table 2. Nutrient specification of basal and standard diets in Exp. 1 (27 to 33 wk of age) and Exp. 2 (42 to 48 wk of age).

Item (g/kg)	Basal diet	Standard diet
Crude protein	120	120
Digestible lysine	4.32	8.30
Digestible total sulphur amino acids	3.60	7.30
Digestible threonine	3.36	5.80
Digestible tryptophan	0.97	1.75
Digestible isoleucine	3.31	6.50
Digestible leucine	10.0	10.0
Digestible arginine	4.52	8.60
Digestible valine	4.35	7.40
Digestible histidine	2.51	2.51
Digestible phenylalanine	4.15	4.15
Digestible tyrosine	3.07	3.07
Digestible proline	7.09	7.09
Digestible alanine	5.28	5.28
Digestible aspartic acid	6.27	6.27
Digestible glutamic acid	16.5	16.5
Digestible glycine	6.14	6.14
Digestible serine	7.48	7.48
Calcium	42.0	42.0
Available phosphorus	4.30	4.30
Dietary electrolyte balance (meq/kg)	220	220
AME (MJ/kg)	11.9	11.9

Table 3. Concentrations of non-bound amino acid and respective specifications of digestible amino acid concentrations used in assay diets (g/kg) in Exp. 1 and Exp. 2.

Amino acids	Dietary digestible amino acid concentrations				
	0% (Basal)	33%	67%	100% (Standard)	133%
Lysine	4.32	5.65	6.97	8.30	9.62
Total sulphur amino acids	3.60	4.83	6.07	7.30	8.53
Threonine	3.36	4.17	4.99	5.80	6.61
Tryptophan	0.97	1.23	1.49	1.75	2.01
Isoleucine	3.31	4.37	5.44	6.50	7.56
Arginine	4.52	5.88	7.24	8.60	9.95
Valine	4.35	5.36	6.38	7.40	8.41
Non-bound amino acids inclusions					
<i>l</i> -lysine HCl	-	2.04	4.08	6.12	8.16
<i>dl</i> -methionine	-	1.24	2.49	3.73	4.97
<i>l</i> -threonine	-	0.85	1.70	2.55	3.40
<i>l</i> -tryptophan	-	0.32	0.64	0.97	1.29
<i>l</i> -isoleucine	-	1.06	2.12	3.19	4.25
<i>l</i> -arginine	-	1.32	2.64	3.96	5.27
<i>l</i> -valine	-	1.05	2.10	3.15	4.20

The assessed amino acid was limiting in the basal diet whilst other amino acid concentrations were equivalent to standard diet.

one standard diet formulated to breeder standard or in total, there were 29 experimental diets.

Diets were based on maize, soybean meal, and canola meal and their nutrient specifications were evaluated by near-infrared spectroscopy using the AMINOIR Advanced programme (Evonik operations GmbH, Hanau, Germany) prior to feed formulation. Each diet was mixed separately and offered in mash form. Amino acid concentrations in diets were determined by 24-h liquid hydrolysis at 110°C in 6 M HCl and then 16n amino acids were analyzed using the Waters AccQTag Ultra chemistry (Waters) on a Waters Acquity UPLC (Milford, MA, USA). The analyzed total amino acid concentrations in standard diets for Exp 1 and Exp 2 are shown in Table 4 and the analyzed total concentrations of test amino acids in all experimental diets are shown in Table 5.

Birds and Housing

A total of 348, twenty five-wk-old Isa Brown laying hens were allocated to individual battery cages (wired-bottom) equipped with individual nipple drinker and trough feeder. Each diet was replicated 6 times with 2 adjacent single cages (2 birds) considered as one replicate. All birds were kept in an environmentally controlled layer shed where temperature, lighting, and ventilation were set according to Isa Brown CS Management guidelines. Exp 1 was conducted from 27 to 33 wk and Exp 2 was carried out from 42 to 48 wk. The assay diets were offered 2 wk prior to data collection period (25–27 wk in Exp 1, 40–42 wk in Exp 2) as an adaptation period. During this period, birds were monitored for egg production and those that were not laying, were replaced. At the end of Exp 1 birds were offered a standard commercial diet formulated to Isa Brown nutrient specification for the relevant age, and then the same

Table 4. Analysed crude protein and amino acid concentrations (total and non-bound) in standard diets in Exp. 1 (27 to 33 wk of age) and Exp. 2 (42 to 48 wk of age).

Item (g/kg)	Exp 1	Exp 2
Crude protein	127	131
Total amino acid		
Lysine	8.62	8.62
Total sulphur amino acids	7.42	7.64
Threonine	6.63	5.94
Tryptophan	1.63	1.61
Isoleucine	6.39	6.51
Leucine	11.3	11.2
Arginine	8.63	9.21
Valine	8.03	7.72
Histidine	2.79	2.77
Phenylalanine	4.71	4.57
Proline	7.74	7.67
Alanine	6.38	6.01
Aspartic acid	7.82	7.40
Glutamic acid	18.5	17.5
Glycine	6.71	6.74
Serine	7.07	6.78
Supplemented non-bound amino acid		
<i>l</i> -Lysine	4.68	5.27
<i>dl</i> -Methionine	3.87	3.92
<i>l</i> -Threonine	2.73	2.97
<i>l</i> -Tryptophan	0.76	0.75
<i>l</i> -Valine	3.25	3.38

birds were re-randomized at the beginning of Exp 2 at wk 40. Diets in both experiments were based on the same raw ingredients.

Experimental Design and Diet Formulation

Data Collection The experimental diets were offered ad-libitum from 25 to 33 wk in Exp 1 (peak of production) and 40 to 48 wk of age in Exp 2 (post peak of production). The first 2 wk of each experiment was considered as an adaptation period; and data were not recorded during this period of time. In both experiments, egg production was recorded daily and egg weights were measured at the end of each week from eggs laid within 48 h prior to weighing. Feed consumption was measured at the end of each Exp on a replicate basis. Dietary

Table 5. Analyzed total concentrations of test amino acids in all experimental diets.

Amino acids	Experiment 1				
	0% (Basal)	33%	67%	100% (Standard)	133%
Lysine	4.01	5.43	6.41	8.25	8.72
Total sulphur amino acids	4.26	5.06	6.7	7.54	9.66
Threonine	3.78	4.18	4.81	6.32	7.26
Tryptophan	4.76	5.64	6.77	7.97	8.27
Isoleucine	3.88	4.73	5.41	6.47	6.53
Arginine	1.00	1.25	1.33	1.60	1.70
Valine	5.65	6.59	7.50	8.94	9.79
Experiment 2					
Lysine	3.93	5.71	6.87	8.99	11.2
Total sulphur amino acids	3.93	4.92	6.12	7.53	8.57
Threonine	3.68	4.49	5.36	6.25	6.62
Tryptophan	4.75	5.48	6.68	7.78	8.50
Isoleucine	3.63	4.63	5.65	6.44	7.30
Arginine	0.91	1.12	1.30	1.65	1.78
Valine	5.41	6.29	7.53	8.90	9.72

Table 6. Estimated digestible amino acid requirements (mg/bird/day) for laying hens determined using linear (LBL) and quadratic broken line (QBL) models based on egg mass, egg production rate, and feed conversion ratio in Exp. 1 (27 to 33 wk of age).

Amino acid	Model	Egg mass (g egg/bird/day)			Egg production rate (%)			Feed conversion ratio		
		Equation	R ²	Requirement	Equation	R ²	Requirement	Equation	R ²	Requirement
Lys	LBL	$y = 58.3 - 0.08(x - 739)$	0.90	739	$y = 95.8 - 0.13(x - 688)$	0.86	688	$y = 2.07 + 0.004(x - 693)$	0.76	693
	QBL	$y = 57.9 - 10^{-4}(x - 917)^2$	0.91	917	$y = 95.5 - 2 \times 10^{-4}(x - 865)^2$	0.87	865	$y = 2.08 + 10^{-4}(x - 865)^2$	0.79	865
TSAA	LBL	$y = 56.5 - 0.09(x - 614)$	0.92	614	$y = 93.2 - 0.14(x - 598)$	0.88	598	$y = 2.08 + 0.003(x - 653)$	0.75	653
	QBL	$y = 58.1 - 10^{-4}(x - 865)^2$	0.93	865	$y = 94.0 - 2 \times 10^{-4}(x - 774)^2$	0.90	774	$y = 2.11 + 10^{-4}(x - 792)^2$	0.73	792
Thr	LBL	$y = 57.7 - 0.10(x - 524)$	0.78	524	$y = 95.0 - 0.17(x - 501)$	0.76	501	$y = 2.11 + 0.017(x - 362)$	0.60	362
	QBL	$y = 57.2 - 3 \times 10^{-4}(x - 585)^2$	0.85	585	$y = 94.8 - 5 \times 10^{-4}(x - 559)^2$	0.83	559	$y = 2.10 + 10^{-4}(x - 393)^2$	0.65	393
Val	LBL	$y = 57.5 - 0.09(x - 648)$	0.85	648	$y = 95.8 - 0.24(x - 527)$	0.91	527	$y = 2.06 + 0.005(x - 537)$	0.68	537
	QBL	$y = 57.2 - 2 \times 10^{-4}(x - 733)^2$	0.88	733	$y = 95.7 - 5 \times 10^{-4}(x - 652)^2$	0.90	652	$y = 2.04 + 10^{-4}(x - 654)^2$	0.68	654
Ile	LBL	$y = 58.1 - 0.08(x - 666)$	0.80	666	$y = 96.8 - 0.12(x - 642)$	0.77	642	$y = 2.12 + 0.007(x - 488)$	0.58	488
	QBL	$y = 57.6 - 10^{-4}(x - 793)^2$	0.83	793	$y = 95.1 - 2 \times 10^{-4}(x - 721)^2$	0.81	721	$y = 2.12 + 10^{-4}(x - 598)^2$	0.62	598
Trp	LBL	$y = 57.6 - 0.36(x - 154)$	0.84	154	$y = 96.3 - 0.60(x - 147)$	0.85	147	$y = 2.02 + 0.008(x - 164)$	0.58	164
	QBL	$y = 57.2 - 0.002(x - 198)^2$	0.85	198	$y = 96.4 - 0.003(x - 188)^2$	0.83	188	$y = 2.00 + 10^{-4}(x - 213)^2$	0.59	213
Arg	LBL	$y = 57.4 - 0.09(x - 640)$	0.52	640	$y = 94.6 - 0.26(x - 534)$	0.55	534	$y = 2.06 + 0.001(x - 733)$	0.18	733
	QBL	$y = 57.3 - 2 \times 10^{-4}(x - 706)^2$	0.57	706	$y = 95.4 - 4 \times 10^{-4}(x - 692)^2$	0.68	692	$y = 2.07 + 10^{-4}(x - 789)^2$	0.21	789

Significance levels for all responses are $P < 0.001$.

In the model, y (output) = L (Max/Min) + U (Slope ratio) \times (x - requirement) for linear broken line; or y (output) = L (Max/Min) + U (Slope ratio) \times (x - requirement)² where L is value at the breaking point/plateau, U = slope ratio of line at $X < R$ and R = value of X at the breaking point/plateau. The estimates of this model are valid if $X < R$; when $X \geq R$, then $Y = \text{Max/Min}$.

amino acid intake for 6 wk was calculated by using daily feed consumption (g/bird/day) and digestible amino acid concentration (g/kg) in each assay diet;

Amino acid consumption

= *Average feed intake*

\times *Digestible amino acid concentration in diet*

Egg production data and egg weights was used to calculate egg mass in each replicate using the following equation;

Egg mass = *Average egg weight*

\times *Average egg production*

Feed conversion ratio in laying chickens were defined as the feed intake required to produced 1g of egg mass;

Feed conversion ratio = *Daily feed intake/Egg mass*

Statistical Analyses

Two birds housed in individual cages were considered as one experimental unit where each amino acid array had 30 observations (including 4 doses plus the standard diet with 6 replicates) for each performance parameter. Linear and quadratic broken line regressions were performed on egg production rate, egg mass and FCR by using the Nutritional Response Model (Version 1.1) developed by Vedenov and Pesti (2008) to estimate the amino acid requirements in each array and statistical significance was accepted at a probability level of 5%.

RESULTS

There was no mortality in Exp. 1 and a mortality of 0.57% was recorded in Exp 2, which was not influenced by treatment ($P > 0.05$).

The estimated digestible amino acid requirements (mg/bird/day) for laying hens determined by linear and quadratic broken line models on maximum egg mass, maximum egg production rate, and minimum FCR in Exp. 1 (27–33 wk of age) is shown in Table 6. There were significant ($P < 0.001$) linear and quadratic broken line regressions obtained for all seven tested amino acids. The highest R² values were observed for TSAA requirement for egg mass and Lys showed highest R² for FCR, whereas the regression for Val had the highest R² value for egg production rate. Arginine exhibited the lowest R² values for all performance parameters. Egg mass and egg production rate provided higher R² values than FCR for all test amino acids. Quadratic broken line generated higher amino acid requirements than linear broken line which indicated an average of 21% increase for egg mass, 23% increase for egg production rate, and 20% increase for FCR. Daily intake estimations of Lys (739 and 917 mg/bird/day), Thr (524 and 585 mg/bird/day), Val (648 and 733 mg/bird/day), and Ile (666 and 793 mg/bird/day) for maximum egg mass were higher than egg production rate and FCR in both linear and quadratic broken line models, as shown in parentheses. The daily intake requirements of Trp and Arg for FCR were higher than other performance parameters regardless of regression models. With linear broken line model, the requirement of TSAA was higher for FCR than egg mass and egg production rate (653 mg/bird/day); whereas, in quadratic broken line model prediction, the requirement for egg mass was higher than other performance parameters (865 mg/bird/day). Regardless of the type of regression models where their predictions were shown in parentheses for linear and quadratic broken line models, Lys (598 and 774 mg/bird/day), TSAA (598 and 774 mg/bird/day), Val (527 and 652 mg/bird/day), Trp (147 and 188 mg/bird/day), and Arg (534 and 692 mg/bird/day) had the lowest requirements for egg production rate compared to egg mass and FCR, whereas Thr (362 and 393 mg/bird/day) and Ile (488 and 598 mg/bird/day) had the lowest requirement for

Table 7. Estimated digestible amino acid requirements (mg/bird/day) for laying hens determined using linear (LBL) and quadratic broken line (QBL) models based on egg mass, egg production rate, and feed conversion ratio in Exp. 2 (42 to 48 wk of age).

Amino acid	Model	Egg mass (g egg/bird/day)			Egg production rate (%)			Feed conversion ratio		
		Equation	R ²	Requirement (mg/d)	Equation	R ²	Requirement (mg/d)	Equation	R ²	Requirement (mg/d)
Lys	LBL	$y = 56.7 - 0.09(x-701)$	0.95	701	$y = 95.1 - 0.14(x-675)$	0.96	675	$y = 2.12 + 0.006(x-592)$	0.80	592
	QBL	$y = 56.2 - 10^{-4}(x-877)^2$	0.97	877	$y = 95.3 - 2 \times 10^{-4}(x-845)^2$	0.97	845	$y = 2.15 + 10^{-4}(x-727)^2$	0.80	727
TSAA	LBL	$y = 56.3 - 0.12(x-565)$	0.95	565	$y = 94.7 - 0.19(x-559)$	0.94	559	$y = 2.14 + 0.007(x-495)$	0.75	495
	QBL	$y = 56.7 - 2 \times 10^{-4}(x-697)^2$	0.94	697	$y = 95.4 - 3 \times 10^{-4}(x-685)^2$	0.94	685	$y = 2.17 + 10^{-4}(x-620)^2$	0.75	620
Thr	LBL	$y = 55.9 - 0.11(x-468)$	0.83	468	$y = 95.1 - 0.20(x-450)$	0.85	450	$y = 2.17 + 0.002(x-446)$	0.43	446
	QBL	$y = 56.1 - 2 \times 10^{-4}(x-605)^2$	0.87	605	$y = 95.6 - 3 \times 10^{-4}(x-609)^2$	0.88	609	$y = 2.20 + 10^{-4}(x-521)^2$	0.51	521
Val	LBL	$y = 57.1 - 0.09(x-627)$	0.93	627	$y = 95.1 - 0.17(x-579)$	0.94	579	$y = 2.10 + 0.002(x-571)$	0.52	571
	QBL	$y = 57.3 - 2 \times 10^{-4}(x-747)^2$	0.94	747	$y = 95.9 - 3 \times 10^{-4}(x-705)^2$	0.94	705	$y = 2.08 + 10^{-4}(x-688)^2$	0.58	688
Ile	LBL	$y = 56.2 - 0.10(x-575)$	0.91	575	$y = 93.5 - 0.16(x-558)$	0.90	558	$y = 2.11 + 0.004(x-515)$	0.71	515
	QBL	$y = 57.1 - 10^{-4}(x-757)^2$	0.92	757	$y = 95.1 - 2 \times 10^{-4}(x-745)^2$	0.91	745	$y = 2.15 + 10^{-4}(x-576)^2$	0.73	576
Trp	LBL	$y = 57.3 - 0.30(x-157)$	0.88	157	$y = 94.9 - 0.52(x-150)$	0.91	150	$y = 2.11 + 0.010(x-131)$	0.53	131
	QBL	$y = 56.9 - 0.002(x-195)^2$	0.91	195	$y = 94.8 - 0.003(x-188)^2$	0.93	188	$y = 2.14 + 10^{-4}(x-163)^2$	0.58	163
Arg	LBL	$y = 56.3 - 0.10(x-586)$	0.51	586	$y = 93.6 - 0.16(x-587)$	0.56	587	$y = 2.01 + 0.001(x-649)$	0.16	649
	QBL	$y = 56.3 - 2 \times 10^{-4}(x-680)^2$	0.59	680	$y = 93.9 - 4 \times 10^{-4}(x-695)^2$	0.62	695	$y = 2.00 + 10^{-4}(x-776)^2$	0.25	776

Significance levels for all responses are $P < 0.001$.

In the model, y (output) = L (Max/Min) + U (Slope ratio) \times (x - requirement) for linear broken line; or y (output) = L (Max/Min) + U (Slope ratio) \times (x - requirement)² where L is value at the breaking point/plateau, U = slope ratio of line at $X < R$ and R = value of X at the breaking point/plateau. The estimates of this model are valid if $X < R$; when $X \geq R$, then $Y = \text{Max/Min}$.

FCR in comparison to egg production rate and egg mass.

The estimated digestible amino acid intake (mg/bird/day) for laying hens in Experiment 2 (42–48 wk of age) is shown in Table 7. Significant ($P < 0.001$) linear and quadratic regression models for egg mass, egg production rate and FCR for all seven amino acids were observed. Both models provided the highest R² values for Lys across all 3 performance parameters; whereas, responses to arginine had the lowest R² values. Both egg mass and egg production rate supported relatively higher R² values than FCR across all 7 amino acids. Similar to Exp 1, quadratic broken line models gave 24.1, 26.9, and 20.1% higher estimations for egg mass, egg production rate and FCR than linear broken line models, respectively. The intake requirement for Lys (701 and 877 mg/bird/day), TSAA (565 and 697 mg/bird/day), Val (627 and 747 mg/bird/day), Ile (576 and 757 mg/bird/day), and Trp (157 and 195 mg/bird/day) were higher for egg mass than egg production rate and FCR. The lowest estimations of amino acid requirements, including Lys, TSAA, Thr, Val, Ile, Trp, and Arg, were recorded for FCR compared to egg mass and egg production rate.

The estimated daily Lys requirements were higher in Exp.1 than Exp.2 for optimal egg mass (linear: 739 vs. 701 mg/bird/day; quadratic: 917 vs. 877 mg/bird/day) egg production rate (linear: 688 vs. 675 mg/bird/day; quadratic: 865 vs. 845 mg/bird/day) and FCR (linear: 693 vs. 592 mg/bird/day; quadratic: 865 vs. 727 mg/bird/day). Similarly, the intake requirements of TSAA were higher in Exp. 1 than Exp. 2, regardless of regression models and production parameters. The intake requirements of Thr in Exp. 1 was higher than Exp. 2 for maximum egg mass with both models (linear: 524 vs. 468 mg/bird/day; quadratic: 585 vs. 605 mg/bird/day) and egg production rate (linear: 501 vs. 450 mg/bird/day; quadratic: 559 vs. 609 mg/bird/day). However, the Thr requirement for minimum FCR was higher in Exp.2 than Exp.1 (linear: 446 vs. 362 mg/bird/day; quadratic: 521 vs. 393 mg/bird/day). The Val requirements for egg

production rate and FCR were higher in Exp. 2 than Exp. 1. Estimated Ile requirements were higher in Exp. 1 with both broken line models for maximum egg mass than Exp. 2 (linear: 666 vs. 576 mg/bird/day; quadratic: 793 vs. 757 mg/bird/day). For egg production rate, Ile requirement was higher in Exp. 1 with linear broken line model (642 vs. 558 mg/bird/day) but was higher in Exp. 2 with quadratic broken line model (745 vs. 721 mg/bird/day). The Ile requirement for FCR was higher in Exp. 2 with linear broken line model (515 versus 488 mg/bird/day) but lower in Exp.2 by quadratic broken line model (598 vs. 576 mg/bird/day). Similarly, there was inconsistent response on Trp and Arg requirements during peak and post-peak production phases and the response depends on regression model and production parameters.

The present study selected egg mass as the parameter to calculate ideal amino acid ratios as R² values derived from their predictive models are higher than those in models predicting FCR. Moreover, egg mass combined the consideration of both the egg production rate and egg weight. The relevant ideal amino acid ratios are tabulated in Table 8. In Exp. 1, the linear broken line model estimated higher ideal amino acid requirements than the quadratic broken line model for Thr (71 vs. 64), Val (88 vs. 80), Ile (90 vs. 86), and Arg (87 vs. 77); whereas quadratic broken line model estimated higher TSAA requirement than the linear broken line model (94 vs. 83). In Experiment 2, the linear broken line model estimated higher ideal amino acid requirements than quadratic broken line models for Val (89 vs. 85) and Arg (84 vs. 78); whereas quadratic broken line models estimated higher ideal amino acid requirements than linear broken line models for Thr (69 vs. 67) and Ile (86 vs. 82).

DISCUSSION

Limited update (Soares et al., 2019) on ideal amino acid recommendations for laying hens was included in the literature since Bregendahl et al. (2008); or ideal

Table 8. Ideal amino acid ratios based on amino acid requirements for maximum egg mass with linear and quadratic broken line models with comparison to [Bregendahl et al. \(2008\)](#) and Isa Brown Breeder Recommendations.

Amino acid	Exp.1 (27 to 33 wk of age)		Exp. 2 (42 to 48 wk of age)		Bregendahl et al. (2008)	Isa Brown Recommendations
	Linear broken line estimation	Quadratic broken line estimation	Linear broken line estimation	Quadratic broken line estimation		
Lysine	100	100	100	100	100	100
TSAA	83	94	81	79	94	87
Threonine	71	64	67	69	77	70
Valine	88	80	89	85	93	88
Isoleucine	90	86	82	86	79	80
Tryptophan	21	22	22	22	22	22
Arginine	87	77	84	78	-	104

amino acid recommendations were reported based on meta-analyses ([Lemme, 2009](#); [Macelline et al., 2021](#)). In [Bregendahl et al. \(2008\)](#), amino acid recommendations for Leghorn-type laying hens were estimated on maximum egg mass by using single-slope broken-line regression model using the nonlinear modeling option in JMP (version 6.0.3; SAS Institute, Cary, NC). In that study, dietary Lys requirement of 538 mg/bird/day was used to calculate ideal amino acid recommendations for Met (47), TSAA (94), Thr (77), Trp (22), Ile (79), and Val (93) on a true digestible basis where the values are shown in parentheses.

There are inconsistencies in amino acid requirements determined by different statistical models. Broken line models are more favorable for estimating amino acid requirements to calculate ideal amino acid ratios while curvilinear models such as exponential and quadratic models are better suited to establish the AA requirements for optimal performance ([Mack et al., 1999](#)). In [Vedenov and Pesti \(2008\)](#), two types of broken line models were used and compared by estimating Lys requirement for weight gain in broiler chickens. A higher estimation for optimal weight gain was reported with quadratic broken line model than with linear broken line model (8.62 vs. 7.50 g/kg). Moreover, 3 types of predictive models (quadratic polynomial, linear broken line, and quadratic broken line) were used in [Liu et al. \(2019\)](#) to estimate the Lys requirement in broiler chickens and requirements were inconsistent among models.

The amino acid requirements obtained in the present study were higher than [Bregendahl et al. \(2008\)](#), for instance, Lys recommendations were higher by 37.4% (739 vs. 538 mg/bird/day) and 30.3% (701 vs. 538 mg/bird/day) in Exp. 1 and Exp. 2, respectively, for egg mass with linear broken line model. Moreover, average optimal egg mass and egg production rates were higher in the present study than [Bregendahl et al. \(2008\)](#). It is worth mentioning that Hy-Line white laying hens were used in [Bregendahl et al. \(2008\)](#) whereas Isa Brown layers were used in the present study. The genetic progression in modern laying hens may also have led to this observation ([Macelline et al., 2021](#)). Moreover, the basal diets used in the 2 studies were quite different in relation to non-bound amino acid inclusions. In the present study, non-bound amino acid inclusions in basal diets were 7.51 and 8.70 g/kg in Experiments 1 and 2,

respectively (L-leucine, L-histidine, glycine, and L-serine). In contrast, previously mentioned amino acids were not supplemented in [Bregendahl et al. \(2008\)](#). The impact of dietary non-bound amino acid on protein digestive dynamics and laying performance was discussed in [Macelline et al. \(2021\)](#). Layer may respond to supplementing non-bound amino acids inefficiently and balanced protein-bound and non-bound amino acids could be beneficial to maintain layer performance.

The concept of ideal amino acid ratio was introduced to minimize the impact of dietary, environment and genetic factors on absolute amino acids requirements ([Baker and Han, 1994](#)) with economically feasible solutions ([Mack et al., 1999](#)). In the present study, average ideal amino acid ratios obtained from linear and quadratic models for egg mass are presented in [Table 8](#) and they were compared with [Bregendahl et al. \(2008\)](#) and Isa Brown Breeder recommendations. The variation of ideal amino acid ratios may be due to breed where [Bregendahl et al. \(2008\)](#) used Hy-Line W-36 laying hens. Ideal amino acid ratios for TSAA, Thr, and Val were higher in [Bregendahl et al. \(2008\)](#) than the present study whereas the ideal ratio of Ile was higher in the present study. Moreover, ideal Ile ratios in Exp. 1 and Exp. 2 are higher than Isa Brown recommendations, whereas Arg ratios from both Experiments noticeably lower than Isa Brown recommendations. The ideal ratio of TSAA was higher in Exp. 1 of the present study with quadratic broken line model while lower in other instance than Isa Brown recommendations. Ideal ratios of Thr in the present study are more comparable with Isa Brown recommendations except in Exp. 2 where quadratic broken line model estimated lower requirement. The inconsistency of predicted ideal amino acid ratios in the present study and literature emphasized the necessity of regularly updating amino acid requirements in laying hens.

The impact of age on amino acid requirements in laying hens was reviewed in [Macelline et al. \(2021\)](#) where there was no clear indication that aging reduces amino acid requirements in laying hens. Usually, amino acid requirements in laying hens are expressed as daily amino acid intake and the present study indicated that the effect of age on amino acid requirements are confounded by regression models ([Table 6](#)). Indeed, only the requirements of Lys and TSAA were higher during 27 to 33 wk of age compared to 42 to 48 wk. This may suggest that

Lys and TSAA are critical amino acids for laying hens at the peak laying stage. However, it is necessary to consider the effect of age on egg shell quality and egg size, especially at post-peak production period (Petricevic et al., 2017) and it cannot be concluded from the present study whether age plays an important role on amino acid requirements during the entire production-cycle.

In the regression models, R^2 can be used as an indicator to discuss the variation of responses to changes of amino acid concentrations in the diets. The lower R^2 value in a regression model suggests that differences between the observed values and the predicted values are wider and amino acid responses are more likely to be biased by experimental error. Moreover, majority of quadratic broken line models in the present study obtained higher R^2 than that of linear broken line model. In the present study, FCR showed the lowest R^2 in amino acid estimations compared to egg mass and egg production rate. Similarly, Bregendahl et al. (2008) also reported similar pattern with all tested amino acids except Arg whose response was not significant. In laying hens, egg production rate, egg weight, and feed intake all contribute to the calculation of FCR and the response of feed intake to different dietary amino acids concentrations are highly variable (Macelline et al., 2021); therefore, FCR may not be an indicative parameter to estimate amino acid requirements in laying hens.

One-way ANOVA was conducted to test whether amino acid intakes in Exp. 1 are significantly different than Exp.2. However, there was no significant difference in amino acids intakes between Exp. 1 and Exp. 2 in all 7 assays ($P > 0.05$) which suggests that age periods used in the present study did not influence amino acid intake in laying hens. The response to age may be more pronounced at post-peak production period. Similarly, based on the Isa Brown CS guide, daily feed intake objective is constant after 27 wk to 100 wk (115 g/day) where cumulative feed intakes between 27 to 33 wk and 42 to 48 are 4.8 kg/bird and 4.7 kg/bird, respectively.

It is interesting that broken line models for Arg was significant with all 3 performance parameters and this was not the case in Bregendahl et al. (2008). This could be due to the Arg concentration in the basal diet (6.90 g/kg) in Bregendahl et al. (2008) already exceeded the requirement. However, in the present study, dietary Arg concentration in the basal diet was 4.35 g/kg which allows responses to changed Arg levels to be observed. However, it is worth noticing that Arg regression models generated the lowest R^2 values compared to other amino acids in the present study. Balnave and Barke (2002) re-evaluated the Arg: Lys antagonism in poultry and concluded that higher or low dietary Arg: Lys ratio adversely influences poultry performance. Okumura and Mori (1979) reported that phenylalanine and histidine responsible for increasing kidney arginase activity. Despite it is not straightforward, the interactions between amino acids brings further challenges on quantifying amino acid requirements. Conventional titration method considers one amino acid at one time where requirements of the test amino acid may be confounded

by dietary concentrations of other related amino acids. Classic examples include Lys and Arg, branch chain amino acid antagonisms. It was proposed by De Leon et al. (2010) and demonstrated by Kidd et al. (2021) that multivariate designs including response surface designs may be introduced to solve this challenge.

CONCLUSIONS

The dietary Lys recommendation was 720 mg/bird/day with linear broken line model and 897 mg/bird/day with quadratic broken line model based on the average values of both experiments. Ideal amino acid ratios relative to Lys for TSAA, Thr, Val, Ile, Trp, Arg were 82, 69, 89, 86, 22, and 86 with linear broken line model and 87, 67, 83, 86, 22, and 78 with quadratic broken line model, respectively. Quadratic broken line model generated higher amino acid requirements and best fits in most of the cases than linear broken line model. Egg mass and egg production rate may be more valid performance parameters than FCR to estimate amino acid requirements.

ACKNOWLEDGMENTS

The feeding study was financially supported by Evonik Evonik Operations GmbH. The authors wish to acknowledge Ms Joy Gill, Ms Kylie Warr, Mr Duwei Chen and Mr Peter Bird (Poultry Research Foundation) and Mr Adam Crawford (Birling Avian Laboratories) for their invaluable technical support. Also, we would like to acknowledge the financial support provided by Australian Government Research and Training Program (International) Scholarship (RTP) for the PhD candidature of Mr Shemil Macelline.

DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

REFERENCES

- Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. *Poult. Sci.* 73:1441–1447.
- Balnave, D., and J. Barke. 2002. Re-evaluation of the classical dietary arginine: lysine interaction for modern poultry diets: a review. *Worlds Poult. Sci. J.* 58:275–289.
- Bregendahl, K., S. Roberts, B. Kerr, and D. Hoehler. 2008. 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. *Poult. Sci.* 87:744–758.
- Coon C. and Zhang B. 1999. Ideal Amino Acid Profile for Layers Examined. *Feedstuffs.* 71:13–15.
- De Leon, A. C., M. T. Kidd, and A. Corzo. 2010. Box-Behnken design: alternative multivariate design in broiler nutrition research. *World Poult. Sci. J.* 66:699–706.

- Faria, D. E.d., R. Harms, R. Antar, and G. Russell. 2003. Re-evaluation of the lysine requirement of the commercial laying hen in a corn-soybean meal diet. *J. Appl. Anim. Res.* 23:161–174.
- Harms, R., and G. Russell. 2001. Evaluation of valine requirement of the commercial layer using a corn-soybean meal basal diet. *Poult. Sci.* 80:215–218.
- Ishibashi, T., Y. Ogawa, T. Itoh, S. Fujimura, K. Koide, and R. Watanabe. 1998. Threonine requirements of laying hens. *Poult. Sci.* 77:998–1002.
- Leeson, S., and J. Summers. 2005. *Commercial Poultry Nutrition*. 3rd ed. Department of Animal and Poultry Science University of Guelph, Ontario, Canada.
- Lemme, A. 2009. Amino acid recommendations for laying hens. *Lohmann Inf.* 44:21–32.
- Liu, S., S. Rochell, C. Maynard, J. Caldas, and M. Kidd. 2019. Digestible lysine concentrations and amino acid densities influence growth performance and carcass traits in broiler chickens from 14 to 35 days post-hatch. *Anim. Feed Sci. Technol.* 255:114216.
- Kidd, M. T., F. Poernama, T. Wibowo, C. W. Maynard, and S. Y. Liu. 2021. Dietary branched-chain amino acid assessment in broilers from 22 to 35 days of age. *J. Anim. Sci. Biotechnol.* 12:6.
- Macelline, S. P., M. Toghyani, P. V. Chrystal, P. H. Selle, and S. Y. Liu. 2021. Amino acid requirements for laying hens: a comprehensive review. *Poult. Sci.* 100:101036.
- Mack, S., D. Bercovici, G. De Groote, B. Leclercq, M. Lippens, M. Pack, J. Schutte, and S. Van Cauwenberghe. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. *Br. Poult. Sci.* 40:257–265.
- NRC. 1994. *Nutrient Requirements of Poultry: 1994*. National Academies Press.
- Okumura, J. I., and S. Mori. 1979. Effect of deficiencies of single essential amino acids on nitrogen and energy utilisation in chicks. *Br. Poult. Sci.* 20:421–429.
- Petricevic, V., Z. Skrbic, M. Lukic, M. Petricevic, V. Doskovic, S. Rakonjac, and M. Marinkovic. 2017. Effect of genotype and age of laying hens on the quality of eggs and egg shells. *Sci. Papers-Ser. D-Anim. Sci.* 60:166–170.
- Schutte, J., J. De Jong, and H. Bertram. 1994. Requirement of the laying hen for sulfur amino acids. *Poult. Sci.* 73:274–280.
- Schutte, J., and W. Smink. 1998. Requirement of the laying hen for apparent fecal digestible lysine. *Poult. Sci.* 77:697–701.
- Soares, L., N. K. Sakomura, J. C.d. P. Dorigam, F. Liebert, A. Sunder, M. Q.d. Nascimento, and B. B. Leme. 2019. Optimal in-feed amino acid ratio for laying hens based on deletion method. *J. Anim. Physiol. Anim. Nutr.* 103:170–181.
- Spangler, H., P. Utterback, C. Parsons, and P. Tillman. 2019. Determining the digestible lysine requirement of 22-to 47-week-old Lohmann laying hens using an increasing protein titration methodology. *Poult. Sci.* 98:1706–1715.
- Vedenov, D., and G. M. Pesti. 2008. A comparison of methods of fitting several models to nutritional response data. *J. Anim. Sci.* 86:500–507.