

# Amino acid requirements for laying hens: a comprehensive review

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**ABSTRACT** The main aim of this review is to consolidate the relevant published data examining amino acid requirements of layer hens and to reach a new set of recommendation based on these data. There are inconsistencies in lysine, sulphur-containing amino acids, threonine, tryptophan, branched-chain amino acids, and arginine recommendations in data that have surfaced since 1994. This review finds that breed, age, basal diet composition, and assessment method have contributed toward inconsistencies in amino acid recommendations. Presently, the development of

reduced-protein diets for layer hens is receiving increasing attention because of the demand for sustainable production. This involves quite radical changes in diet composition with inclusions of nonbound, essential and nonessential amino acids. Increasing inclusions of nonbound amino acids into layer diets modifies protein digestive dynamics, and it may influence amino acid requirements in layer hens. This review considers present amino acid recommendations for layer hens and proposes refinements that may better serve the needs of the layer industry in the future.

**Key words:** amino acid requirement, layer hen, nonbound amino acid, protein digestive dynamic

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## INTRODUCTION

There has been a substantial global expansion in egg production by the layer industry as the volume of egg production has increased by 119% (35.5 vs. 76.8 million tonnes) from 1990 to 2018 (<http://www.fao.org>) (Food and Agricultural Organization of the United Nations, 2018). This growth represents an average annual increase of 2.84%. China produced 458 billion eggs in 2018, and the USA produced 109 billion eggs in 2018 (<https://www.statista.com>). China had the highest per capita egg consumption (22.7 kg) in 2017, followed by Japan (19.6 kg) and Mexico (19.3 kg) (<https://www.ourworldindata.org>) (Our world in data Per capita egg consumption in world, 2020). Increasing global egg production is inevitable because eggs are considered as an

imperative protein source for humans, and the world population is forecast to increase by 24% from 7.8 billion in 2020 to 9.7 billion in 2050 (<https://www.worldometers.info>) (Woldometers). Eggs are a balanced source of nutrients because they contain 457 g/kg protein on dry matter basis (Lunven et al., 1973) with all the essential amino acids, omega-3 fatty acids, key vitamins (A, D, E, B<sub>12</sub>), minerals, and antioxidants (Sparks, 2006; Browning and Cowieson, 2014). The balanced nutrient composition of eggs is specifically recommended during pregnancy and for juvenile brain development. Moreover, hens transfer dietary vitamin D into egg yolks efficiently such that egg yolks contain both vitamin D<sub>3</sub> and high levels of 25-hydroxyvitamin D<sub>3</sub> (Browning and Cowieson, 2014). Osteoporosis is a major human health problem, and consequently the high levels of 25-hydroxyvitamin D<sub>3</sub> in eggs are beneficial (Van Den Bergh et al., 2011).

The increasing demand for eggs has generated challenges to the layer industry including food security, food safety, feed ingredient shortages, disease problems, and increasing productions costs and environmental issues (Wang et al., 2017). The provision of diets closely matching optimum requirements is a key strategy to

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overcome these challenges by optimising raw material usages, lowering production costs, and attenuating the loss of nutrients in effluent, including nitrogen and phosphorus, released into the environment.

Amino acids and energy density are 2 critical components in the least-cost feed formulations for layers; however, amino acid requirements will vary depending on breed, age, feeding strategies, housing conditions, and the accuracy with which requirements have been assessed. Moreover, genetic improvements and enhanced performance of laying hens have modified the optimum inputs of amino acid and energy, which are interdependent. The increasing availability of nonbound (synthetic or crystalline) amino acids demands a better appreciation of amino acid levels in layer diets simply because their digestive dynamics differ from protein-bound amino acids (Liu and Selle, 2017). Perhaps insufficient attention has been paid to amino acid requirements in layers since NRC (1994) as subsequent outcomes are somewhat inconsistent. This review consolidates the relevant data in a comprehensive manner. The purpose of this review is to identify the shortfalls and to recommend directions that research should be taken in the future.

## Methodology

The studies selected in this review were completed since 1994, and the impact of single amino acids on performance of laying hens was determined in some; although standard statistical models were applied to estimate amino acid recommendations in the remaining studies. Where data permit, the impacts of dietary amino acids concentrations on feed intake are tabulated. Reported amino acid recommendations based on various performance parameters are also tabulated. Feed conversion ratio (FCR) and egg mass were identified as the appropriate parameters to derive amino acid recommendations in laying hens because they reflected egg production, egg weight, and feed conversion to egg production. Therefore, the simple mean recommendations of egg mass and FCR were used to summarise amino acid recommendations reported during the past 30 yr (Table 16), and relevant age ranges for the recommendations are included as footnotes. Standard deviations of the mean amino acid recommendations are recorded to illustrate the variations caused by assessment method, breed, and diet composition.

Various methods of assessment have been used in recent studies to arrive at amino acid recommendations in laying hens; these include linear, quadratic, and polynomial broken-line regressions, exponential models, and second order functions. Broken-line regressions are established models to arrive at amino acid recommendations. There were 8 studies (Peganova et al., 2003; Saki et al., 2012; Kakhki et al., 2016a, 2016b; Mousavi et al., 2018; Wen et al., 2019a, 2019b; Spangler et al., 2019) in which more than one assessment method was applied; therefore, it is possible to compare the impacts that different methods have on amino acid

recommendations. Both linear and quadratic broken-line assessments were used in 5 studies (Kakhki et al., 2016a, 2016b; Mousavi et al., 2018; Wen et al., 2019a, 2019b), and on average, quadratic broken-line models generated 15.2% higher recommendations than linear broken-line models. Alternatively, polynomial regressions generated 23.1% higher recommendations than linear broken-line models in 4 studies (Mousavi et al., 2018; Spangler et al., 2019; Wen et al., 2019a, 2019b).

## AMINO ACID PROFILE OF EGGS

Yolk protein is continually synthesised in the liver, whereas albumin protein is synthesised in the magnum on a discontinuous basis (Edwards et al., 1976; Carvalho et al., 2018). Essentially, egg yolk, albumin, and eggshell with membrane make up 30, 60, and 10% of the total egg by weight, respectively (Chambers et al., 2017). Crude protein contents in eggs are shown in Table 1. From Table 1, it appears that crude protein content in egg yolk is usually higher than that in egg white on an as-is basis, whereas egg white has a higher crude protein content than egg yolk on the dry matter basis. Low-density lipoproteins, high-density lipoprotein, phosvitin, and livetin are the major proteins in egg yolk, and ovalbumin, ovotransferrin, ovomucoid, ovomucin, ovoglobulin, and lysozyme are the major protein groups in egg white (Guha et al., 2018).

The analyzed amino acid profile of eggs reported in the literature is shown in Table 2; glutamic acid is the dominant amino acid while leucine is the major essential amino acid. There is, however, little information in the literature about the impact of dietary amino acids on egg amino acid profiles, and the relevant data are ambiguous. The amino acid composition in eggs varies with breed, age, diet, and environmental settings (Nimalaratne et al., 2011; Attia et al., 2020; Mori et al., 2020); however, some studies have reported that these factors do not alter the amino acid composition in eggs (Lunven et al., 1973; Secci et al., 2018; Attia et al., 2020). This disparity may have arisen from differences in the analytical procedures used. However, Martín-Venegas et al. (2011) reported that higher sulphur-containing amino acids (methionine and cysteine) in diets reduced leucine levels in eggs, and Carvalho et al. (2018) reported that dietary methionine levels did not change methionine egg concentrations but sulphur-containing amino acids did have linear negative impacts on concentrations of branched-chain amino acids (BCAA; isoleucine, leucine, valine), histidine, and proline in eggs.

## AMINO ACID RECOMMENDATIONS FOR LAYER HENS

The amino acid recommendations for layer hens are not consistent; the genesis of these inconsistencies could be genetic differences between breeds and the method by which amino acid requirements were assessed. Usually,

**Table 1.** Crude protein contents in edible egg fractions.

Basis	Reference	Breed	Dietary Crude protein, (g/kg)	Crude protein, (g/kg)		
				Whole egg	Egg white	Egg yolk
Dry matter	Lunven et al. (1973)	White leghorn	110	464	845	31.5
	Lunven et al. (1973)	White leghorn	200	474	852	31.2
	Lunven et al. (1973)	New Hampshire	110	441	840	28.5
	Lunven et al. (1973)	New Hampshire	200	450	829	30.7
	Freschi et al. (2011)	Na	-	-	-	23.0
	Carvalho et al. (2018)	Lohmann LSL	170	537	-	-
As-is	Donadelli et al. (2019)	Na	-	500	-	-
	Li-Chan (1989)	Na	-	-	100-110	-
	Secci et al. (2018)	Lohmann brown	180	-	112	16.0
	Secci et al. (2018)	Lohmann brown	180	-	111	15.6
	Attia et al. (2020)	Commercial lines	-	139	124	16.0

Abbreviation: Na, not available.

breed-specific amino acid guidelines are adopted in practice as the dietary requirements for layer hens. The amino acid requirements recommended by various breeder companies are presented in Table 3; interestingly, there are tangible differences across the guidelines.

Amino acid requirements for leghorn-type layers were published by NRC (1994) and are still considered one of main references for amino acid recommendations. However, genetic improvements and higher performance objectives set by the layer industry have motivated researchers to review the NRC (1994) recommendations over the past 26 yr. Consequently, a number of recent studies have been published; however, different experimental methodologies have been used. In addition, several experiments were conducted in which the requirement of one single amino acid was determined (Schutte and Smink, 1998; Faria et al., 2003; Gomez and Angeles, 2009; Wen et al., 2019b).

The concept of ideal amino acid ratios was introduced for swine (NRC, 1998) and broilers (Emmert and Baker, 1997; Mack et al., 1999) to facilitate least-cost feed

formulations. This approach was adopted for layers, and 5 such amino acid profiles are shown in Table 4. Most of these profiles were compiled from different amino acid assays under various experimental conditions with the exception of the study by Bregendahl et al. (2008). Importantly, a similar study has not since been completed; therefore, further studies are required to update ideal amino acid profiles in laying hens.

## IMPORTANCE OF DIETARY AMINO ACIDS IN LAYING HENS

### Lysine

Lysine is the second limiting amino acid in typical maize-soybean meal-based poultry diets, and dietary supplementation of feed-grade lysine was adopted in the 1970s (Kidd et al., 2013). Accurate estimations of lysine requirements are critical because recommendations for the balance of amino acids are expressed as ratios to lysine (Emmert and Baker, 1997), and the

**Table 2.** Amino acid concentrations in eggs.

Amino acid, (g/kg)	Reference				
	Carvalho et al. (2018) (DM basis)	Ali et al. (2019) (DM basis)	Donadelli et al. (2019) (DM basis)	Li et al. (2019) (as-is)	Attia et al. (2020) (as-is)
Arginine	33.5	27.3	32.7	6.10	8.10
Histidine	13.1	12.9	12.9	2.50	3.10
Isoleucine	26.9	22.1	29.3	5.40	6.70
Leucine	45.1	34.2	46.7	8.50	10.9
Lysine	36.9	28.9	41.4	7.50	9.60
Methionine	16.5	16.2	17.4	3.90	3.50
Phenylalanine	30.8	21.2	30.1	6.40	6.50
Phenylalanine	-	37.8	52.8	11.6	11.7
Threonine	24.5	21.8	23.7	5.50	6.40
Tryptophan	-	6.00	8.70	-	1.40
Valine	33.4	27.8	36.5	6.40	7.40
Alanine	29.7	27.8	-	10.3	6.60
Aspartic acid	53.0	47.1	-	9.30	12.8
Cysteine	8.70	6.10	11.4	1.00	2.00
Glutamic acid	67.0	58.5	-	10.3	16.5
Glycine	17.5	16.7	19.1	11.7	4.30
Proline	19.6	15.7	19.0	7.50	5.20
Serine	38.8	33.6	31.4	4.50	12.2
Tyrosine	-	16.6	22.7	5.20	5.20
Total	495	478	436	124	140

**Table 3.** Digestible amino acid intakes in laying hens based on breeder recommendations.

Amino acid, mg/b/d	Hy-line W36 (Hy-line international, 2020) <sup>1</sup>	Hy-line brown (Hy-line International, 2018) <sup>1</sup>	Lohmann brown-lite (Tierzucht, 2017a) <sup>2</sup>	Lohman brown classic (Tierzucht, 2017b) <sup>2</sup>	Isa brown (Isa Brown, 2011) <sup>4,3</sup>
Lysine	800	820	680	720	851
Methionine	420	410	330	360	462
Methionine + Cystine	730	746	610	660	735
Threonine	560	574	470	500	598
Tryptophan	170	172	150	150	191
Arginine	860	853	700	750	
Isoleucine	640	656	540	570	767
Valine	700	722	590	630	819

<sup>1</sup>First egg to production drops 2% below peak egg production (approximately 18–37 wk).

<sup>2</sup>50% egg production to maximum egg production (approximately 21–40 wk).

<sup>3</sup>From 2% eggs production to 28 wk of age.

<sup>4</sup>Based on a feed intake of 105 g/bird/day.

concept of ideal protein ratios is widely applied in least-cost feed formulations. It is for this reason that lysine is considered first. Adequate provision of lysine maintains immune competence and digestive tract functionality in poultry (Kidd 2004; Vaezi et al., 2011). Moreover, increasing dietary lysine have been shown to improve egg production, egg weight, egg mass, and feed conversion efficiency in laying hens (Faria et al., 2002; Kakhki et al., 2016a).

While lysine usually has a positive impact on feed intake, some studies have found reduced feed intakes as a consequence of high level of dietary lysine (Bregendahl et al., 2008). It does appear that diets containing more than 8.0 g/kg lysine depressed or had no impact on feed intake (Table 5). However, there is a positive correlation between the total dietary lysine levels and feed intake in hens ( $r = 0.566$ ,  $P < 0.0001$ ) from 40 observations in studies completed since 1996 (Table 5).

Lysine requirements, expressed as daily intakes (mg/bird/day), are presented in Table 6. In this review, egg mass and FCR are considered to be the most indicative parameters for egg production. The highest recommendation was reported by Kakhki et al. (2016a), and the lowest recommendation was made by Bregendahl et al. (2008). The major differences between these 2 studies were crude protein levels in the basal diet (123 vs. 155 g/kg) and the statistical models used. Moreover, a wider range of dietary lysine (5.1–12.4 g/kg) was tested by Bregendahl et al. (2008) than that (6.6–8.6 g/kg) by Kakhki et al. (2016a), and these researchers reported greater increases in feed intake. As shown in Table 6, the

highest variation (CV of 18%) in lysine recommendations was observed for egg mass, and the lowest variation (CV of 10%) was detected for the FCR. Furthermore, the lowest average digestible lysine recommendation was for egg production (691 mg/b/d), whereas the highest value was found for FCR (751 mg/b/d). On the basis of egg mass and FCR, simple means of daily total and digestible lysine intakes are 717 and 730 mg/bird/day, respectively, based on studies completed since 1998 (Table 6).

### Sulphur-Containing Amino Acids

Methionine and cysteine, the total sulphur-containing amino acids (TSAA), are the first limiting AA in practical poultry diets because of their limited presence in protein sources of plant origin (Schutte and Van Weerden, 1978). Methionine receives more focus than cysteine in poultry because it is responsible for cellular metabolism as a methyl donor, formation of coenzymes, and acts as the precursor to cysteine (Bunchasak, 2009). In 1951, methionine was the first feed-grade amino acid introduced into poultry diets, and it was adopted rapidly (Kidd et al., 2013). Even now, TSAA requirements are often met by supplementing nonbound methionine rather than considering methionine and cysteine in tandem. Also, there is high methionine + cysteine in protein synthesis for feathering (Fisher et al., 1981). However, the optimal methionine-to-cysteine ratio remains problematic, and Baker et al. (1996) reckoned that cysteine should not exceed 0.52% of TSAA content in poultry diets. Cysteine proportions of TSAA have become lower more recently, and standard maize-soybean meal diets

**Table 4.** Proposed ideal digestible amino acids ratios in diets for laying hens.

Amino acid	Reference				
	Coon and Zhang (1999)	Leeson and Summers (2005)	Rostagno et al. (2005)	Bregendahl et al. (2008)	Lemme, (2009)
Lysine	100	100	100	100	100
Methionine	49	51	50	47	50
Methionine + Cystine	81	88	91	94	91
Threonine	73	80	66	77	70
Tryptophan	20	21	23	22	21
Arginine	130	103	100	-	104
Isoleucine	86	79	83	79	80
Valine	102	89	90	93	88

**Table 5.** Effects of total and digestible dietary lysine concentrations on significant responses in feed intakes of laying hens.

Basis	Reference	Age (weeks)	Breed	Dietary lysine (g/kg)	Dietary energy (MJ/kg)	Response (g/bird/d)
Total	Faria et al. (2003)	44-51	Hy-line W-36	4.8 vs. 5.6	12.29 vs. 12.18	80.7 vs. 94.1
	Faria et al. (2003)	44-51	Hy-line W-36	5.6 vs. 7.6	12.18 vs. 12.07	NS
	Faria et al. (2003)	58-65	Hy-line W-36	5.2 vs. 6.8	12.25 vs. 12.03	NS
	Faria et al. (2003)	58-65	Hy-line W-36	5.6 vs. 8.0	12.19 vs. 11.86	86.1 vs. 93.5
	Bouyeh and Gevorgian (2011)	52-64	Hy-line W-36	5.6 vs. 6.9	11.99	NS
	Bouyeh and Gevorgian (2011)	52-64	Hy-line W-36	7.6 vs. 5.6	11.99	101.0 vs. 113.3
	Rama Rao et al. (2011)	21-72	Babcock white leghorn	6.5 vs. 8.0	10.89	NS
	Prochaska et al. (1996)	23-38	Hy-line W-36	13.7 vs. 8.9	11.51	85.0 vs. 93.0
	Prochaska et al. (1996)	23-38	Hy-line W-36	7.2 vs. 11.5	11.51	NS
	Prochaska et al. (1996)	42-64	Hy-line W-36	7.0 vs. 15.8	11.43	NS
Digestible	Schutte and Smink (1998)	24-36	White leghorn	4.9 vs. 7.7	11.76	NS
	Bregendahl et al. (2008)	28-34	Hy-line W-36	5.1 vs. 6.9	12.51	77.8 vs. 89.0
	Bregendahl et al. (2008)	28-34	Hy-line W-36	8.7 vs. 6.9	12.51	80.6 vs. 87.9
	Bregendahl et al. (2008)	52-58	Hy-line W-36	3.4 vs. 7.2	12.18	78.2 vs. 97.3
	Rocha et al. (2009)	24-40	Hy-line W-36	5.5 vs. 7.7	11.77 vs. 11.85	93.2 vs. 99.7
	Cupertino et al. (2009)	54-70	Light laying	5.6 vs. 7.6	11.72 vs. 11.76	102.0 vs. 113.0
	Cupertino et al. (2009)	54-70	Brown type	5.6 vs. 7.6	11.72 vs. 11.76	NS
	Santos et al. (2014)	28-44	Isa Brown	6.0 vs. 7.5	-	NS
	Santos et al. (2014)	28-44	Isa Brown	9.0 vs. 7.5	-	100.5 vs. 116.8
	Nunes et al. (2015)	50-66	Shaver Brown	7.9 vs. 9.1	11.93	NS
	Pastore et al. (2015)	60-76	Hy-line W-36	6.0 vs. 9.0	12.14 vs. 12.17	NS
	Kakhki et al. (2016a)	32-44	Hy-line W-36	6.6 vs. 7.6	12.14	93.1 vs. 101.1
	Kakhki et al. (2016a)	32-44	Hy-line W-36	8.1 vs. 8.6	12.14	NS
	Pastore et al. (2018)	24-40	Hy-line W-36	6.0 vs. 9.0	12.14 vs. 12.17	91.1 vs. 96.7
	Spangler et al. (2019)	22-47	Lohmann LSL-Lite	5.8 vs. 7.5	12.14	NS
	Spangler et al. (2019)	22-47	Lohmann LSL-Lite	5.1 vs. 8.5	12.14	75.7 vs. 103.2

Abbreviation: NS, nonsignificant.

may meet cysteine requirement for laying hens without additional cysteine (Harms and Russell, 1996).

The effects of TSAA on feed intakes in laying hens in studies from 1996 are summarised in Table 7. It has been suggested that dietary TSAA are capable of regulating the appetite of the animals (Fisher and Boorman, 1984), and several studies have found that feed intakes were linearly increased by elevating dietary TSAA in laying hens (Brumano et al., 2010; Carvalho et al., 2018). However, excess dietary methionine concentrations have been shown to depress feed intakes in laying hens despite the overall increased methionine intake (Shafer et al., 1996, 1998). While some studies have found positive impacts of TSAA on feed intakes, a negative correlation ( $r = -0.289$ ,  $P = 0.015$ ) was found between the dietary TSAA and feed intake across 71 data points from studies since 1996 as tabulated in Table 7.

TSAA recommendations taken from the literature are presented in Table 8. Most of the studies reported their recommendations on a total basis rather than on a digestible basis of amino acids. The highest TSAA requirement for optimising egg mass and FCR was reported in the study by Narváez-Solarte et al. (2005) as opposed to the lowest requirement documented in the study by Saki et al. (2012). Interestingly, these 2 studies had large differences in feed intake and choice of statistical models for their assessments. The highest TSAA recommendation was made for egg weight, and the lowest for weight gain of laying hens (Table 8). Moreover, reports on TSAA requirements in the literature for egg weight had the highest variation (CV of 24%), and FCR had the lowest variation (CV of 16%). As shown in Table 16, TSAA requirements averaged

603 mg/bird/6day on a total basis and 621 mg/bird/day when expressed in a digestible basis. However, these calculated values are not theoretically acceptable because total basis values always should be higher than digestible values, and this reflects the inconsistency in reporting of TSAA recommendations for layer hens. Moreover, the difference of mean age range for the simple means of TSAA recommendations in digestible basis is 32 to 46 wk, whereas the mean age range for total basis is 22 to 37 wk which may also have had an impact on these inconsistent outcomes.

### Threonine

Threonine is considered as the third limiting amino acid in conventional poultry diets (Fernandez et al., 1994), especially in layer diets containing less than 140 g/kg crude protein (Azzam et al., 2017). Feed-grade threonine became commercially available in 1980s (Kidd et al., 2013), and it is routinely included in diets for pigs and poultry. Threonine is important in poultry because it is prominent in intestinal mucin secretion and in production of antibodies (Cardoso et al., 2014a). Hence, threonine plays a major role in immunity, and the requirement could be elevated under challenge conditions including poor sanitation and chronic diseases.

Many studies have reported positive impacts of adequate and excess provision of dietary threonine on layer performance (Martinez-Amezcuca et al., 1999; Faria et al., 2002; Abdel-Wareth and Esmail, 2014), while some studies did not detect any responses (Gomez and Angeles, 2009; Azzam et al., 2011). Effects of dietary threonine on feed intake reported in the

**Table 6.** Total and digestible lysine recommendations for laying hens.

Reference	Breed	Age, (weeks)	Statistical model	Dietary CP, (g/kg)	Predicted egg mass (g/bird/ d)	Total lysine recommendations, (mg/bird/d)				
						Weight gain	FCR	Egg production	Egg weight	Egg mass
Schutte and Smink, (1998)	White leghorn	24-36	Exponential curve <sup>1</sup>	164	57		900			534
Faria et al. (2003)	Hy-line W-36	44-51	Linear broken line	107-149				633		
Faria et al. (2003)	Hy-line W-36	58-65	Linear broken line	112-153				606		
Mean		46.3		141.5			900			534
						Digestible lysine recommendations, (mg/bird/day)				
						Weight gain	FCR	Egg production	Egg weight	Egg mass
Schutte and Smink (1998)	White leghorn	24-36	Exponential curve	164	57		720			540
Bregendahl et al. (2008)	Hy-line W-36	28-34	Linear broken line	123	49	490	693	482	649	538
Bregendahl et al. (2008)	Hy-line W-36	52-58	Linear broken line	96	49		690	511	573	508
Cupertino et al. (2009)	Light laying	54-70	Polynomial regression	143	48		764	787	767	784
Cupertino et al. (2009)	Brown type	54-70	Polynomial regression	143	48		748	742		748
Rocha et al. (2009)	Hy-line W-36	24-40	Polynomial regression	145	-		759			759
Figueiredo et al. (2012)	Hy-line W-36	42-58	Polynomial regression	160	54		805		766	780
Santos et al. (2014)	Isa Brown	28-44	Polynomial regression	136-170		876				
Silva et al. (2015)	Dekalb White	37-40	Polynomial & broken line <sup>2</sup>	69-224				707		
Silva et al. (2015)	Dekalb White	41-44	Polynomial & broken line <sup>2</sup>	69-224				660		
Silva et al. (2015)	Dekalb White	45-48	Polynomial & broken line <sup>2</sup>	69-224				669		
Kakhki et al. (2016a)	Hy-line W-36	32-44	Linear broken line	155	55			814	778	810
Kakhki et al. (2016a)	Hy-line W-36	32-44	Quadratic broken line	155	55			848	828	843
Pastore et al. (2018)	Hy-line W-36	24-40	Polynomial regression	150	-			780	820	800
Spangler et al. (2019)	Lohmann LSL	22-47	Linear broken line	139-217	-		690	528		655
Spangler et al. (2019)	Lohmann LSL	22-47	Polynomial regression	139-217	-		886	727		817
Mean		41.3		148.9			751			715

Simple mean of total lysine recommendations based on feed conversion ratio (FCR) and egg mass is  $717 \pm 258.8$  mg/g/bird. Simple mean of digestible lysine recommendations based on FCR (SD =  $\pm 64.4$ ) and egg mass (SD =  $\pm 121.9$ ) is  $730 \pm 100.8$  mg/g/bird.

$$^1 Y = a + b[1 - e^{-c(x-d)}]$$

<sup>2</sup>Linear broken line.

literature were inconsistent as shown in Table 9. However, it has been found that threonine levels less than 4.2 g/kg tended to reduce feed intake of layers (Faria et al., 2002; Bregendahl et al., 2008).

Threonine recommendations from the literature are presented in Table 10. Similarly, requirements were different based on the parameter selected, and variations in threonine requirements for FCR and egg mass are smaller than those in other parameters tabulated (CV of <6.5%). Based on FCR and egg mass, average total and digestible threonine requirements in laying hens were calculated to be 457 and 486 mg/bird/day, respectively (Table 10). These mean values are somewhat problematic in that it would be expected to have higher recommendations when threonine is expressed on a total basis, and variations in respective age ranges for total

and digestive threonine recommendations may be an influential factor for these outcomes.

## Tryptophan

There are much fewer studies investigating tryptophan requirements for laying hens. Tryptophan plays an important role in generating several metabolites, including serotonin, indoleamine 2,3-dioxygenase, and quinolinic acid (Bai et al., 2017; Khattak and Helmbrecht, 2019). Therefore, tryptophan is involved in reducing stress and increasing appetite in animals (Le Floch and Seve, 2007). Tryptophan, phenylalanine, and tyrosine are aromatic amino acids involved in the synthesis of key neurotransmitters (serotonin,

**Table 7.** Effect of dietary total and digestible sulphur-containing amino acid concentrations on significant responses in feed intakes of laying hens.

Basis	Reference	Age, (weeks)	Breed	Dietary TSAA (g/kg)	Dietary methionine (g/kg)	Dietary energy (MJ/kg)	Response (g/bird/d)
Total	Shafer et al. (1996)	52-60	DeKalb XL		4.0 vs. 3.0	11.96	104.0 vs. 107.0
	Shafer et al. (1998)	32-56	Commercial strain		5.3 vs. 3.8	11.69	105.0 vs. 108.0
	Sohail et al. (2002)	21-33	Hy-Line W-36	6.5 vs. 8.1		11.76	NS
	Ahmad and Roland (2003)	20-36	DeKalb Delta	6.5 vs. 8.1		11.77 vs. 11.74	NS
	Harms and Russell (2003)	47-53	Hy-Line W-36		2.0 vs. 3.0	12.43 vs. 11.72	61.5 vs. 96.7
	Harms and Russell (2003)	47-53	Hy-Line W-36		3.0 vs. 3.4	11.72 vs. 11.44	NS
	Novak et al. (2004)	20-43	DeKalb Delta	6.5 vs. 9.2	3.6 vs. 6.2	12.10	NS
	Bunchasak and Silapasorn (2005)	22-44	Isa-brown		2.6 vs. 3.0	-	87.9 vs. 98.2
	Bunchasak and Silapasorn (2005)	22-44	Isa-brown		3.0 vs. 4.4	-	NS
	Narvaez-Solarte, (2005)	22-38	Lohmann white	4.9 vs. 7.3		11.51	91.9 vs. 108.1
	Gomez and Angeles (2009)	100-106	Hy-Line W-36		1.9 vs. 4.5	12.14	92.5 vs. 98.5
	Alagawany et al. (2011)	18-34	Lohmann	6.7 vs. 7.7		11.72	NS
	Saki et al. (2012)	22-36	Commercial strain		2.4 vs. 3.4	11.86	64.53 vs. 84.42
	Saki et al. (2012)	22-36	Commercial strain	6.2 vs. 7.7	3.4 vs. 4.9	11.86	NS
	Rama Rao et al. (2011)	21-72	Lohmann white		3.1 vs. 4.0	10.89	NS
	Nassiri Moghaddam et al. (2012)	70-76	Hy-Line W-36		2.7 vs. 3.4	11.53 vs. 11.56	NS
	Safaa et al. (2008)	56-68	Brown Hy-line		3.1 vs. 3.6	-	NS
Digestible	Bregendahl et al. (2008)	28-34	Hy-Line W-36	3.5 vs. 5.7	1.9 vs. 4.1	12.51	62.7 vs. 86.3
	Bregendahl et al. (2008)	28-34	Hy-Line W-36	7.8 vs. 5.7	6.3 vs. 4.1	12.51	81.6 vs. 86.3
	Bregendahl et al. (2008)	52-58	Hy-Line W-36	3.6 vs. 5.9	1.3 vs. 3.7	12.18	60.8 vs. 97.5
	Kakhki et al. (2016b)	32-44	Hy-Line W-36	5.1 vs. 7.1		12.10	NS
	Carvalho et al. (2018)	20-50	Lohmann white	4.7 vs. 6.5		11.23	99.3 vs. 102.3

Abbreviations: NS, nonsignificant; TSAA, total sulphur-containing amino acids.

dopamine, noradrenaline) which influence the behavior of poultry (Wurtman et al., 1980; Fernstrom and Fernstrom, 2007). Moreover, tryptophan is a serotonin precursor, and low serotonin levels in brain tissue lead to aggressive feather-pecking and cannibalism in layers resulting in substantial production losses (Birkl et al., 2017). Indoleamine 2,3-dioxygenase and quinolinic acid play vital roles in maintaining immune functions (Bai et al., 2017) and metabolism of carbohydrates and lipids (Khattak and Helmbrecht, 2019). Finally, tryptophan plays a rate-limiting role in protein synthesis because of its lowest concentrations in organisms in comparison to the balance of amino acids (Corzo et al., 2005).

It is considered that conventional layer diets contain sufficient tryptophan to meet requirements (Mousavi et al., 2018); however, supplementation of feed-grade tryptophan may become necessary in diets with reduced crude protein and increased levels of feed-grade amino acids. Moreover, Dong et al. (2012) reported that laying hens offered 150 g/kg CP diets and from 0.2 to 0.4 g/kg non-bound tryptophan displayed improved eggshell strength and immune functions in birds under hot and humid conditions. In contrast, excess inclusions of tryptophan may have negative impacts on layer performance as fermentation of tryptophan in caeca generates deleterious end products, including indole and skatole which hinder ATP mitochondrial formation (Khattak and Helmbrecht, 2019).

The effects of tryptophan on feed intake are summarised in Table 11. Dietary tryptophan has positive impacts on feed intake, but results are too inconsistent to provide any estimations. However, it has been reported that layer hens offered diets with 1.56 g/kg or lower levels of tryptophan showed depressed feed intake (Mousavi et al., 2018; Wen et al., 2019a). However, Table 11 shows that dietary tryptophan was

positively correlated with feed intake in layer hens ( $r = 0.554$ ,  $P = 0.0012$ ). Table 12 summarized tryptophan recommendations from the literature since 1999. Most recommended requirements were reported on a digestible basis, and predictions were mainly based on egg production, egg mass, and FCR. Only one study reported the tryptophan requirement based on egg weight (Lima et al., 2012), and another 2 reported nonsignificant effects of dietary tryptophan on egg weight (Dong et al., 2012; Mousavi et al., 2018). However, higher variations ( $CV > 21\%$ ) in tryptophan requirements can be detected for egg production, egg mass, and FCR. It seems that lower tryptophan levels are required to optimize FCR than those needed for optimal egg production and egg mass. In this review, digestible and total tryptophan requirements were calculated as 149 and 178 mg/bird/day, respectively (Table 16).

### Branched-Chain Amino Acids

It is important to formulate diets with a balanced profile of BCAA to maintain performance of layer hens because antagonisms between BCAA (isoleucine, leucine, valine) exist and have been reported to depress performance (Peganova and Eder, 2002; Azzam et al., 2015). Valine and isoleucine are the most examined BCAA, while leucine attracts less attention as leucine requirements are usually met by the various protein sources in conventional layer diets. Recently, feed-grade valine and isoleucine inclusions became more feasible economically, and more accurate estimations of BCAA requirements become more necessary. In addition to protein synthesis, adequate supplies of valine and isoleucine are important to maintain gut immunity, tight junctions, antioxidant capacity, and unique metabolic

**Table 8.** Total and digestible sulphur-containing amino acids recommendations for laying hens.

Reference	Breed	Age (weeks)	Statistical model	Dietary CP (g/kg)	Predicted egg mass (g/bird/d)	TSAA recommendations (mg/bird/d)					Total methionine recommendations (mg/bird/d)					
						Weight gain	FCR	Egg production	Egg weight	Egg mass	Weight gain	FCR	Egg production	Egg weight	Egg mass	
Harms and Russel (2003)	Hy-Line W-36	47-53	Broken line <sup>1</sup>	93.4-198									246	311		
Harms and Russel (2003)	Hy-Line W-36	47-53	Broken line <sup>1</sup>	123-229									246	189		
Novak et al. (2004)	Dekalb Delta	20-43	Polynomial	177			699	811	877							
Novak et al. (2004)	Dekalb Delta	44-63	Polynomial	179-180					779							
Narvaez-Solarte (2005)	Leghorn	22-38	Polynomial	144	57		751	733	724	749	730					
Saki et al. (2012)	Commercial	22-36	Exponential <sup>2</sup>	153	-		578	610	489	602	610	299	388	239	388	388
Saki et al. (2012)	Commercial	22-36	Second order <sup>3</sup>	153	44		554	560	531	586	558	329	338	310	363	337
Saki et al. (2012)	Commercial	22-36	Broken line <sup>1</sup>	153	42		475	461	470	620	469	255	244	229	303	249
Mean		37.8		160.2				613			592		323			325
						Digestible SAA recommendations (mg/bird/day)					Digestible methionine recommendations (mg/bird/day)					
						Weight gain	FCR	Egg production	Egg weight	Egg mass	Weight gain	FCR	Egg production	Egg weight	Egg mass	
Bregendahl et al. (2008)	Hy-Line W-36	28-34	Broken line <sup>1</sup>	123	49	498	462	511	264	506	153	253		143	253	
Bregendahl et al. (2008)	Hy-Line W-36	52-58	Broken line <sup>1</sup>	96	51		690	690	626	488		268		370	264	
Gomez and Angeles (2009)	Hy-Line W-36	100-106	Polynomial	140								313		343		
Kakhki et al. (2016b)	Hy-Line W-36	32-44	Broken line <sup>1</sup>	154	56		656	678	641	673						
Kakhki et al. (2016b)	Hy-Line W-36	32-44	Broken line <sup>4</sup>	154	56		706	710	697	726						
Carvalho et al. (2018)	Lohman white	20-50	Quadratic	167-175	-	621	652	646	662	655						
Mean		49.7		139.7			633			610		261			259	

Simple means of total sulphur amino acids recommendations based on FCR (SD = ±109.2) and egg mass (SD = ±109.0) is 603 ± 102.7 mg/g/bird. Simple means of total methionine recommendations based on FCR (SD = ±73.1) and egg mass (SD = ±70.3) is 324 ± 64.2 mg/g/bird. Simple means of digestible sulphur amino acids recommendations based on FCR (SD = ±98.4) and egg mass (SD = ±106.2) is 621 ± 97.3 mg/g/bird. Simple means of digestible methionine recommendations based on FCR (SD = ±10.6) and egg mass (SD = ±7.8) is 260 ± 7.7 mg/g/bird. Simple means of digestible sulphur amino acids recommendations based on FCR (SD = ±98.4) and egg mass (SD = ±106.2) is 621 ± 97.3 mg/g/bird.

Abbreviations: FCR, feed conversion ratio; TSAA, total sulphur-containing amino acids.

<sup>1</sup>Linear broken line.

<sup>2</sup> $Y = a + b[1 - e^{-c(x-d)}]$

<sup>3</sup> $Y = a + bX + CX^2$

<sup>4</sup>Quadratic broken line.



**Table 9.** Effects of dietary total and digestible threonine concentrations on significant responses in feed intakes of laying hens.

Basis	Reference	Age (weeks)	Breed	Dietary threonine (g/kg)	Dietary energy (MJ/kg)	Response (g/bird/d)
Total	Ishibashi et al. (1998)	29-32	DeKalb White Leghorn	3.1 vs. 5.1	12.14	NS
	Ishibashi et al. (1998)	39-47	DeKalb White Leghorn	3.1 vs. 5.1	12.14	NS
	Faria et al. (2002)	34-39	Hy-Line W36	5.3 vs. 4.2	11.96 vs. 12.40	NS
	Faria et al. (2002)	34-39	Hy-Line W36	3.5 vs. 5.0	11.96 vs. 12.40	72.8 vs. 99.7
	Faria et al. (2002)	48-53	Hy-Line W36	4.0 vs. 5.8	11.73 vs. 12.17	NS
	Schmidt et al. (2009)	34-50	Lohman LSL	4.1 vs. 5.2	11.73	117 vs. 112
	Schmidt et al. (2009)	34-50	Lohman brown	4.1 vs. 5.5	11.73	114 vs. 110
	Abdel-Wareth and Esmail (2014)	68-76	Hy-Line Brown	4.7 vs. 6.7	-	95.5 vs. 100.0
	Abdel-Wareth and Esmail (2014)	68-76	Hy-Line Brown	6.7 vs. 10.7	-	NS
	Digestible	Bregendahl et al. (2008)	28-34	Hy-Line W36	3.8 vs. 4.5	12.51
Cupertino et al. (2010)		54-58	Lohman LSL	4.5 vs. 5.1	11.73	107 vs. 111
Cupertino et al. (2010)		54-58	Lohman brown	3.8 vs. 5.1	11.73	NS
Azzam et al. (2011)		40-48	Babcock Brown	4.7 vs. 8.7	12.15	NS
Rocha et al. (2013b)		24-40	Hy-Line W36	4.8 vs. 6.6	12.14	NS
Cardoso et al. (2014a)		60-76	DeKalb White Leghorn	5.2 vs. 6.2	11.72	108.2 vs. 110.1
Azzam et al. (2019)		28-40	Lohman brown	4.3 vs. 7.4	11.93	NS

Abbreviation: NS, nonsignificant.

processes in poultry (Azzam et al., 2015; Dong et al., 2016; Wen et al., 2019b). Moreover, BCAA regulate fatty acid metabolism in the liver (Bai et al., 2015), which may be important in egg production because hepatic yolk-lipoprotein production may be a rate-limiting factor for egg formation.

The effect of dietary valine and isoleucine on feed intake in laying hens is presented in Table 13. Most studies show significant impacts of valine and isoleucine on feed intakes, although the results are inconsistent. It was reported that valine deficiency reduced feed intakes of laying hens especially when dietary valine inclusions were lower than 7.3 g/kg (Wen et al., 2019b). Moreover, increasing dietary digestible valine linearly increased feed intakes of laying hens as reported by Lelis et al. (2014). Moreover, dietary isoleucine levels lower than 4.0 g/kg and higher than 8.1 g/kg may have negative impacts on feed intake in layer hens (Peganova and Eder, 2002; Shivazad et al., 2002). In the present review, dietary valine was positively correlated to feed intake ( $r = 0.742$ ,  $P < 0.0001$ ), while there were no correlations between dietary isoleucine and feed intake (Table 13).

Variations in BCAA recommendations are summarized in Tables 14 and 15 however, very few studies were designed to investigate the interactive effects of BCAA on individual requirements. BCAA antagonisms in these studies may have influenced the tabulated outcomes. On average, the highest estimation of valine and isoleucine were reported for egg mass and FCR, respectively. Higher variations in isoleucine requirements were observed for egg weight, egg production, and egg mass. The highest valine recommendation was reported by Wen et al. (2019b), and they used higher isoleucine contents in their basal diet than other studies. The highest isoleucine estimation for FCR and egg mass were reported by Bregendahl et al. (2008) and Rocha et al. (2013a), respectively. On average, valine requirements were calculated as 614 and 532 mg/bird/day on a total and

digestible basis, respectively. However, there are inconsistencies for isoleucine because the estimation made on a digestible basis was higher than that made on a total basis (437 and 648 mg/bird/day) as shown in Table 15.

## Arginine

Arginine is one of the critical essential amino acids for poultry because, unlike mammals, they cannot synthesise arginine (Birmani et al., 2019); however, arginine has received little consideration in layer hens. Arginine is required to enhance the performance and immunity in layers as it serves as a precursor of protein, creatine, proline, polyamines, and nitric oxide (Lieboldt et al., 2015).

Increasing dietary arginine has improved feed intakes, but it has negative effects at levels of more than 12.7 g/kg (Yuan et al., 2015) although arginine can act as appetite-stimulating factor in layer hens (Lieboldt et al., 2015). In addition, arginine can stimulate the secretion of luteinizing hormone and positively impact follicle development and ovulations (Youssef et al., 2015; Yuan et al., 2015). Interestingly, arginine is a powerful secretagogue which increases the release of insulin, growth hormones, and insulin-like growth factor-I (Silva et al., 2012). Furthermore, the beneficial effect of 14.4 g/kg arginine on intestinal villus development has been reported in layers by Yu et al. (2018). However, high dietary arginine may result in impaired performance because it interacts with lysine absorption because of competition between these 2 amino acids for access to specific intestinal transporter systems (Closs et al., 2004; Yu et al., 2018).

Few studies have examined arginine requirement in layers; however, layer hens have higher arginine requirements of 920 and 870 mg/bird/day in the early laying period according to the specific breeder guidelines of Hy-line and Lohmann, respectively, than for other essential amino acids. Based on egg mass, total arginine recommendation was estimated at 760 mg/bird/day in the study by Leeson and Summers (2005) for 32 to 45 wk. Moreover, inconsistent outcomes such as

**Table 10.** Total and digestible threonine recommendations for laying hens.

Reference	Age (weeks)	Breed	Statistical model	Dietary CP (g/kg)	Predicted egg mass (g/bird/d)	Total threonine requirement (mg/bird/d)				
						Weight gain	FCR	Egg production	Egg weight	Egg mass
Ishibashi et al. (1998)	29-32	DeKalb White Leghorn	Broken line <sup>1</sup>	144	54		456			453
Ishibashi et al. (1998)	29-32	DeKalb White Leghorn	Broken line <sup>1</sup>	144	53		467			457
Faria et al. (2002)	34-39	Hy-Line W36	Broken line <sup>2</sup>	97-144	53			439		462
Faria et al. (2002)	48-53	Hy-Line W36	Broken line <sup>2</sup>	116-160	47			394		447
Mean	37			137			462			455
						Digestible threonine requirement (mg/bird/day)				
						Weight gain	FCR	Egg production	Egg weight	Egg mass
Schmidt et al. (2009)	34-50	Lohman LSL	Polynomial				510			
Schmidt et al. (2009)	34-50	Lohman brown	Polynomial				505			
Bregendahl et al. (2008)	28-34	Hy-Line W36	Broken line <sup>2</sup>	123	50	390	461	400	418	414
Cupertino et al. (2010)	54-58	Lohman LSL	LRP	131	51			489		487
Cupertino et al. (2010)	54-58	Lohman brown	LRP	131	50			508		505
Rocha et al. (2013b)	24-40	Hy-Line W36	Polynomial	142			523	523	606	
Azzam et al. (2019)	28-40	Lohman brown	Polynomial	140				708		
Mean	41.9			133			500			469

Simple mean of total threonine recommendations based on FCR (SD =  $\pm 7.8$ ) and egg mass (SD =  $\pm 6.3$ ) is  $457 \pm 6.9$  mg/g/bird. Simple mean of digestible threonine recommendations based on FCR (SD =  $\pm 26.9$ ) and egg mass (SD =  $\pm 48.2$ ) is  $486 \pm 37.6$  mg/g/bird.

Abbreviation: LRP, linear response plateau.

<sup>1</sup>Combination of linear and quadratic broken lines.

<sup>2</sup>Linear broken line.

digestible arginine recommendations estimated at 968 and 791 mg/bird/day for 33 to 49 and 35 to 47 wk, respectively, based on egg mass were obtained by [Coon and Zhang \(1999\)](#) for Hy-Line W-36 layers. It has become apparent from this review that diet composition may impact arginine requirements because most arginine poultry studies have used high levels of the corn-gluten meal which has high leucine contents, but corn-gluten meal is not frequently used on practice. Therefore, leucine and lysine dietary levels should be considered in future studies to determine arginine requirements more precisely.

## Nonessential Amino Acids

It is assumed that conventional layer diets contain adequate nonessential amino acids; therefore, little attention has been placed on their requirements for layer hens. However, with reduced-crude protein diets, nonessential amino acids assume more importance because less nitrogen is available for their synthesis. Feed-grade nonessential amino acids have yet to become cost-effective in poultry, and some studies have investigated the effect of nonbound, nonessential amino acids on layer performance.

**Table 11.** Effects of dietary total and digestible tryptophan concentrations on significant responses in feed intakes of laying hens.

Basis	Reference	Age (weeks)	Breed	Dietary tryptophan (g/kg)	Dietary energy MJ/kg	Response (g/bird/d)	
Total	<a href="#">Russell and Harms (1999)</a>	53-59	Hy-Line W36	1.1 vs. 1.9	12.13	65.2 vs. 90.8	
	<a href="#">Russel and Harms (1999)</a>	53-59	Hy-Line W36	1.5 vs. 2.3	12.13	NS	
	<a href="#">Harms and Russell (2000b)</a>	28-36	Hy-Line W36	1.4 vs. 1.8	12.08 vs. 11.85	84.9 vs. 92.6	
	<a href="#">Harms and Russel, (2000b)</a>	28-36	Hy-Line W36	1.8 vs. 2.0	11.85 vs. 11.73	NS	
	<a href="#">Harms and Russel (2000b)</a>	28-36	Hy-Line W36	1.2 vs. 1.8	12.18 vs. 11.85	92.6 vs. 68.0	
	<a href="#">Peganova et al. (2003)</a>	31-37	Lohman brown	1.0 vs. 2.0	11.45	87.0 vs. 120.6	
	<a href="#">Peganova et al. (2003)</a>	31-37	Lohman brown	1.3 vs. 2.5	11.45	NS	
	<a href="#">Dong et al. (2012)</a>	40-48	Babcock Brown	1.7 vs. 2.5	10.83	NS	
	<a href="#">Lima et al. (2012)</a>	29-49	White laying hens	1.5 vs. 2.0	12.14	NS	
	<a href="#">Khattak and Helmbrecht (2019)</a>	22-38	Lohman brown	1.0 vs. 3.1	11.83	NS	
	<a href="#">Wen et al. (2019a)</a>	41-60	Hy-Line W36	1.2 vs. 2.0	12.46	85.1 vs. 97.2	
	Digestible	<a href="#">Bregendahl et al. (2008)</a>	28-34	Hy-Line W36	0.9 vs. 1.7	12.51	61.6 vs. 87.9
		<a href="#">Calderano et al. (2012)</a>	24-40	Hy-Line W36	1.6 vs. 1.9	12.56	74.9 vs. 78.1
		<a href="#">Cardoso et al. (2014b)</a>	60-76	Dekalb White	1.7 vs. 2.0	11.72	NS
<a href="#">Mousavi et al. (2018)</a>		30-36	Hy-Line W36	0.8 vs. 1.4	12.23	74.1 vs. 91.6	

Abbreviation: NS, nonsignificant.

**Table 12.** Tryptophan recommendations for laying hens.

Reference	Age (weeks)	Breed	Statistical model	Dietary CP (g/kg)	Predicted egg mass (g/bird/d)	Total tryptophan recommendations (mg/bird/d)				
						Weight gain	FCR	Egg production	Egg weight	Egg mass
Russel and Harms (1999)	53-59	Hy-Line W36	Broken line <sup>1</sup>	132				136		
Harms and Russel (2000b)	28-36	Hy-Line W36	Broken line <sup>1</sup>	113-159				140		
Peganova et al. (2003)	31-37	Lohman brown	Broken line <sup>1</sup>	125	49	164		162		185
Peganova et al. (2003)	31-37	Lohman brown	Broken line <sup>1</sup>	125	49	225		151		151
Peganova et al. (2003)	31-37	Lohman brown	Exponential curve	125	49	192		179		210
Peganova et al. (2003)	31-37	Lohman brown	Exponential curve	125	48	199		162		159
Wen et al. (2019a)	41-60	Hy-Line W36	Broken line <sup>1</sup>	155	49		140	153		156
Wen et al. (2019a)	41-60	Hy-Line W36	Broken line <sup>2</sup>	155	50		164	182		200
Wen et al. (2019a)	41-60	Hy-Line W36	Polynomial	155	50		182	180		187
Wen et al. (2019a)	41-60	Hy-Line W36	Exponential curve	155	54		178	210		226
Means				138.8			166			184
						Digestible tryptophan recommendations (mg/bird/day)				
						Weight gain	FCR	Egg production	Egg weight	Egg mass
Bregendahl et al. (2008)	28-34	Hy-Line W36	Broken line <sup>1</sup>	123	49	145	95	119		120
Calderano et al. (2012)	24-40	Hy-Line W36	LRP	145	50		142			144
Cardoso et al. (2014b)	60-76	Dekalb White	Polynomial	140	60		211	212		212
Lima et al. (2012)	29-49	White laying hens	Polynomial	157	60		196	190	192	192
Mousavi et al. (2018)	30-36	Hy-Line W36	Broken line <sup>1</sup>	140	48		104	114		104
Mousavi et al. (2018)	30-36	Hy-Line W36	Broken line <sup>2</sup>	140	48		118	136		134
Mousavi et al. (2018)	30-36	Hy-Line W36	Polynomial	140	50		162	166		166
Mousavi et al. (2018)	30-36	Hy-Line W36	Exponential curve		?			147		142
Khattak and Helmbrecht (2019)	22-38	Lohman brown	Polynomial	147				250		
Means	36.8			142			147			152

Simple mean of total tryptophan recommendations based on FCR (SD =  $\pm 18.9$ ) and egg mass (SD =  $\pm 27.3$ ) is  $178 \pm 25.5$  mg/g/bird. Simple mean of digestible threonine recommendations based on FCR (SD =  $\pm 44.9$ ) and egg mass (SD =  $\pm 36.3$ ) is  $149 \pm 39.1$  mg/g/bird. Exponential curve.  $Y = a + b[1 - e^{-c(x-d)}]$ .

<sup>1</sup>Abbreviation: LRP, linear response plateau.

<sup>2</sup>Linear broken line.

<sup>3</sup>Quadratic broken line.

Glycine and serine supplementation in reduced-crude protein diets has been shown to improve broiler performance (Siegert et al., 2015); however, overall outcomes are somewhat inconsistent. Glycine may serve as a serine precursor, and the dietary value of both amino acids may be expressed as glycine equivalents (Dean et al., 2006). Glycine is involved in the synthesis of purines, heme groups, glutathione, and creatine (Han and Thacker, 2011); bile salt metabolism (Powell et al., 2009); and greatly in uric acid excretion (Bondi, 1987). However, no glycine and serine dose response or recommendation studies have been published in laying hens. Dietary levels of 10 g/kg glycine or 14.6 to 16.2 g/kg glycine equivalents in diets containing 169 g/kg crude protein have been shown to improve feed intakes, egg weights, and digestibility of fat (Han and Thacker, 2011). Also, the positive impact of glycine supplementation on fat digestibility helped to improve performance in laying hens who offered standard CP diets (Han and Thacker, 2011). Moreover, improved layer performance was elicited by glycine supplementation of a reduced-crude protein

diet, and it was found that 16 g/kg of glycine plus serine optimised egg mass and FCR (Akinde and Etop, 2014).

Glutamic acid is considered to be important as it plays crucial roles in intestinal function and development (D'Mello, 2003; Burrin and Stoll, 2009) and effects the intestinal morphology in poultry (Maiorka et al., 2000). Moreover, glutamic acid is the dominant amino acid in egg protein. However, there is a lack of evidence in the literature to support the beneficial effects of glutamic acid on layer performance and intestinal morphology. However, 23 g/kg glutamic acid has been shown to have positive effects on calcium availability for eggshell and bone mineralization (Pereira et al., 2019). Similarly, improvements in bone growth have followed elevations of dietary glutamic acid (Silva et al., 2001a; Silva et al., 2001b). Fatigue and osteoporosis cause severe production losses in the layer industry, mainly because of the cortical bone loss for high Ca demand in layer hens, and may shorten their production cycle. Therefore, further investigations into the effects of glutamic acid on bone mineralisation in layer hens are justified to extend their production cycle.

**Table 13.** Effects of dietary valine and isoleucine concentrations on significant responses in feed intakes of laying hens.

Basis	Reference	Age (weeks)	Breed	Valine (g/kg)	Dietary energy (MJ/kg)	Response (g/bird/d)
Total	Harms and Russell (2001)	39-	Hy-Line W36	5.3 vs. 7.0	12.22 vs. 11.88	81 vs. 96
	Azzam et al. (2015)	40-47	Hy-Line brown	9.0 vs. 11.0	11.31	115 vs. 110
	Wen et al. (2019b)	41-60	Hy-Line W36	5.2 vs. 7.3	12.46	67 vs. 93
	Lelis et al. (2014)	42-54	Dekalb Brown	5.6 vs. 6.6	12.35	NS
Digestible	Bregendahl et al. (2008)	28-34	Hy-Line W36	4.7 vs. 8.8	12.51	54 vs. 87

  

Basis	Reference	Age (weeks)	Breed	Isoleucine (g/kg)	Dietary energy (MJ/kg)	Response (g/bird/day)
Total	Harms and Russell (2000a)	32-38	Hy-Line W36	4.9 vs. 6.7	12.09 vs. 11.70	NS
	Peganova and Eder (2002)	28-33	Lohmann brown	6.9 vs. 8.1	11.44	102 vs. 87
	Peganova and Eder (2002)	24-33	Lohmann brown	4.3 vs. 10.5	11.44	117 vs. 88
	Peganov, and Eder (2002)	46-54	Lohmann brown	5.1 vs. 10.5	11.44	115 vs. 97
	Shivazad et al. (2002)	37-43	Hy-Line W36	3.9 vs. 5.7	12.31 vs. 11.99	70 vs. 91
	Rocha et al. (2013a)	24-40	Hy-Line W36	5.9 vs. 8.0	12.20	NS
	Dong et al. (2016)	29-40	Lohmann brown	5.4 vs. 9.4	11.26	NS
	Parenteau et al. (2020)	20-27	White Shaver	6.0 vs. 7.5	11.72	NS
	Parenteau et al. (2020)	20-27	White Shaver	5.7 vs. 6.4	11.72	116 vs. 110
	Digestible	Bregendahl et al. (2008)	28-34	Hy-Line W36	3.7 vs. 6.4	12.51

Abbreviation: NS, nonsignificant.

## NONBOUND VS. PROTEIN-BOUND AMINO ACIDS

The acceptance of nonbound or feed-grade amino acids in poultry diet formulations commenced in the 1950s and have become a key factor in least-cost diet formulations. It took 40 yr for the acceptance of feed-grade lysine and threonine which were developed only slowly after the rapid adoption of methionine. A high emphasis has since been placed on identifying the most appropriate inclusion rates of nonbound amino acids in poultry diets (Kidd et al., 2013). The application of nonbound amino acids in layer diets is relatively limited, but their inclusion in reduced-CP layer diets is likely to increase.

Recently, it has been reported that the digestive dynamics of protein-bound and nonbound amino acids

are inherently different in poultry, which impacts their performance (Liu and Selle, 2017; Selle and Liu, 2019). The concept of protein digestive dynamics is described as a combination of protein digestion rates, absorption of amino acids, and their transition across the gut mucosa into the portal circulation (Selle and Liu, 2019). However, this concept has yet to be evaluated in layers as thoroughly as it has been in broiler chickens.

Beneficial effects from nonbound amino acid inclusions in layer diets were reported by Sohail et al. (2002), which demonstrated the advantage of basing diet formulations on amino acids rather than crude protein. Egg weight, egg production, and egg mass responded to inclusions of nonbound lysine, threonine, isoleucine, and tryptophan in layer diets irrespective of dietary protein levels (Sohail et al., 2002). The nonbound amino acid

**Table 14.** Valine recommendations for laying hens.

Reference	Age (weeks)	Breed	Statistical model	Isoleucine (g/kg)	CP (g/kg)	Predicted egg mass (g/bird/d)	Total valine recommendations (mg/bird/d)				
							Weight gain	FCR	Egg production	Egg weight	Egg mass
Harms and Russel (2001)	39	Hy-Line W36	Broken line <sup>1</sup>	6.0-7.0	113-159			593	678		
Wen et al. (2019b)	41-60	Hy-Line W36	Broken line <sup>1</sup>	6.6	134	49	501	592		597	
Wen et al. (2019b)	41-60	Hy-Line W36	Broken line <sup>2</sup>	6.6	134	49	650	691		720	
Wen et al. (2019b)	41-60	Hy-Line W36	Polynomial	6.6	134	49	648	698		675	
Wen et al. (2019b)	41-60	Hy-Line W36	Exponential	6.6	134	49	549	634		574	
Means	50.3			6.58	134.4		587			642	

  

Digestible valine recommendations (mg/bird/d)											
Reference	Age (weeks)	Breed	Statistical model	Isoleucine (g/kg)	CP (g/kg)	Predicted egg mass (g/bird/d)	Digestible valine recommendations (mg/bird/d)				
							Weight gain	FCR	Egg production	Egg weight	Egg mass
Bregendahl et al. (2008)	28-34	Hy-Line W36	Broken line <sup>1</sup>	4.1	123	49	520	477	493	517	501
Lelis et al. (2014)	42-54	Dekalb Brown	Polynomial	5.5	148			591	597	502	
Lelis et al. (2014)	42-54	Dekalb Brown	LRP	5.5	148			560	563		
Means	42			5.03	139.7			543			501

Simple mean of total tryptophan recommendations based on FCR (SD =  $\pm 74.2$ ) and egg mass (SD =  $\pm 67.9$ ) is  $614 \pm 72.0$  mg/g/bird. Simple mean of digestible tryptophan recommendations based on FCR (SD =  $\pm 58.9$ ) and egg mass is  $532 \pm 52.4$  mg/g/bird. Exponential curve.  $Y = a + b[1 - e^{-c(x-d)}]$

Abbreviation: LRP, linear response plateau.

<sup>1</sup>Linear broken line.

<sup>2</sup>Quadratic broken line.

**Table 15.** Isoleucine recommendations for laying hens.

Reference	Age (weeks)	Breed	Statistical model	Valine (g/kg)	CP (g/kg)	Predicted egg mass (g/hen/d)	Total isoleucine recommendations (mg/bird/d)				
							Weight gain	FCR	Egg production	Egg weight	Egg mass
Harms and Russell (2000)	38-44	Hy-Line W36	Broken line <sup>1</sup>	6.0-8.0	125-164				589	601	
Peganova and Eder (2002)	25-32	Lohmann brown	Exponential	5.9	121	57					412
Peganova and Eder (2002)	24-32	Lohmann brown	Exponential	6.4	139	53					436
Peganova and Eder (2002)	46-54	Lohmann brown	Exponential	6.4	139	56					431
Shivazad et al. (2002)	37-43	Hy-Line W36	Broken line <sup>1</sup>	5.0-7.0	99-142	50			450	497	469
Means	38.0			6.34	132.8						437
							Digestible isoleucine recommendations (mg/bird/d)				
Bregendahl et al. (2008)	28-34	Hy-Line W36	Broken line <sup>1</sup>	5.3	123	48	490	693	427	649	538
Rocha et al. (2013a)	24-40	Hy-Line W36	Polynomial	7.5	163	42		681			681
Means	31			6.40	143.0			687			610

Simple mean of total isoleucine recommendations based on egg mass is  $437 \pm 23.7$  mg/g/bird. Simple mean of digestible isoleucine recommendations based on FCR (SD =  $\pm 8.5$ ) and egg mass (SD =  $\pm 101.1$ ) is  $648 \pm 73.7$  mg/g/bird.

<sup>1</sup>Quadratic broken line.

supplementation of reduced-crude protein diets to meet required levels has maintained egg mass, egg production, and FCR in laying hens to match those offered conventional diet (Ji et al., 2014; Rojas et al., 2015). Not surprisingly, the addition of nonbound lysine to conventional layer diets did not change the performance of layer hens (Sohail et al., 2003). Interestingly, the capacity of broiler breeders to use nonbound amino acids efficiently has been questioned by Nonis and Gous (2006).

However, it has been reported that a reduced-CP diet supplemented with nonbound methionine, lysine, tryptophan, glycine, and glutamic acid reduced albumin protein synthesis and lowered egg weight compared to hens fed conventional diet (Penz and Jensen, 1991). Therefore, it may be that nonbound amino acids do not influence protein synthesis in the magnum. Interestingly, Osonka et al. (1947) suggested that methionine in

peptide form would influence egg protein content more than nonbound methionine, which indicates that methionine in peptide form may be superior to supply the magnum for 3 h after ovulation. The strategy of supplying higher protein-bound amino acids at the time of albumin protein synthesis was tested by Penz and Jensen (1991), who found a beneficial effect on egg weights. Furthermore, it was considered that an inadequate nitrogen pool in reduced-crude protein diets specially when the ovum is in the magnum might be triggering lower egg weights, although diets were supplemented with an adequate level of nonbound amino acids (Penz and Jensen, 1991; Leeson and Caston, 1996; Ji et al., 2014). Therefore, supplementation of nonbound, nonessential amino acids could be beneficial to maintain layer performance when birds are offered reduced-CP diets.

**Table 16.** NRC (1994) amino acid recommendations on a total basis plus simple means derived from more recent data based on FCR and egg mass on total and digestible bases in laying hens.

Amino acid	Recommendations (g/bird/d)			Ratios relative to lysine		
	NRC (1994)	Derived (total basis)	Derived (digestible basis)	NRC (1994)	Derived (total basis)	Derived (digestible basis)
Lysine	690	$717 \pm 258.8$ (n = 2) <sup>1</sup>	$726 \pm 101.7$ (n = 20) <sup>1</sup>	100	100	100
Methionine	300	$324 \pm 64.2$ (n = 6) <sup>2</sup>	$260 \pm 7.7$ (n = 4) <sup>2</sup>	43	45	36
Methionine + cystine	580	$603 \pm 102.7$ (n = 9) <sup>3</sup>	$621 \pm 97.3$ (n = 10) <sup>3</sup>	84	84	86
Threonine	470	$457 \pm 6.9$ (n = 6) <sup>4</sup>	$486 \pm 37.6$ (n = 7) <sup>4</sup>	68	64	67
Tryptophan	160	$178 \pm 25.5$ (n = 12) <sup>5</sup>	$149 \pm 39.1$ (n = 15) <sup>5</sup>	23	25	21
Valine	700	$614 \pm 72.0$ (n = 8) <sup>6</sup>	$532 \pm 52.4$ (n = 4) <sup>6</sup>	101	86	73
Isoleucine	650	$437 \pm 23.7$ (n = 4) <sup>7</sup>	$648 \pm 73.7$ (n = 4) <sup>7</sup>	94	61	89
Leucine	820			118		
Arginine	700			101		
Phenylalanine + tyrosine	830			120		
Histidine	170			25		

<sup>1</sup>Derived from Table 6 (mean ages ranged from 24-36 and 35-50 wks for total and digestible basis, respectively).

<sup>2</sup>Derived from Table 8 (mean ages ranged from 22-36 and 39-45 wks for total and digestible basis, respectively).

<sup>3</sup>Derived from Table 8 (mean ages ranged from 22-37 and 32-46 wks for total and digestible basis, respectively).

<sup>4</sup>Derived from Table 10 (mean ages ranged from 33-37 and 37-46 wks for total and digestible basis, respectively).

<sup>5</sup>Derived from Table 12 (mean ages ranged from 37-52 and 33-43 wks for total and digestible basis, respectively).

<sup>6</sup>Derived from Table 14 (mean ages ranged from 41-60 and 34-44 wks for total and digestible basis, respectively).

<sup>7</sup>Derived from Table 15 (mean ages ranged from 33-40 and 25-37 wks for total and digestible basis, respectively).

**Table 17.** Simple means of amino acid recommendations derived from more recent data based on FCR and egg mass which separated based on the breed.

Amino acid	Total basis (mg/bird/d)		Digestible basis (mg/bird/d)	
	Leghorn type	Hy-line W-36	Leghorn	Hy-line W-36
Lysine	717 ± 258.8 (24-36 wks)		630 ± 127.3 (44-59 wks)	726 ± 110.8 (35-46 wks)
Methionine + cystine	721 ± 18.8 (21-40 wks)			613 ± 108.9 (35-46 wks)
Threonine	458 ± 6.07 (29-32 wks)	455 ± 10.6 (41-46 wks)		260 ± 7.7 (39-45 wks)
Tryptophan		179 ± 26.7 (41-60 wks)	203 ± 10.2 (45-53 wks)	466 ± 54.7 (28-34 wks)
Valine		614 ± 72.0 (41-60 wks)		130 ± 27.8 (29-37 wks)
Isoleucine		469 (37-43 wks)	426 ± 12.7 (32-39 wks)	489 ± 16.9 (26-34 wks)
				648 ± 73.1 (25-37 wks)
				502 ± 10.1 (41-53 wks)
				576 ± 21.9 (42-54 wks)

## CONCLUSIONS AND IMPLICATIONS

The poultry industry's environmental impact is becoming a major challenge which could result in restrictions being placed on poultry production. Higher nitrogen excretion levels into environment are an increasing issue, and reduced-CP diets have been identified as a potential solution. However, the effects of reducing dietary crude protein on the performance of laying hens are yet to be thoroughly investigated.

Inconsistent results stemming from dietary CP reductions on layer performance have been reported where some studies did not find impaired performance (Liang et al., 2005; Gunawardana et al., 2008; Novak et al., 2008) while others reported underperformance in comparison to control diets (Halle 2002; Valkonen et al., 2006; Alagawany et al., 2020). A lack of attention on protein digestive dynamics in reduced-crude protein layer diets which is important because they are supplemented with higher levels of nonbound amino acids has been observed. Earlier, Osonka et al. (1947) reported that nonbound and protein-bound amino acids effect egg protein synthesis differently. Therefore, it is important to investigate protein digestive dynamics in layers further so that their performance is maintained. Moreover, it is evident that egg weight is the most sensitive parameter to nonbound amino acid supplementation that attracts a focus on the impact of protein digestive dynamic on both albumin and yolk protein synthesis. Alternatively, the low nitrogen pool in birds offered reduced-CP diets could result in an inadequacy of nonessential amino acids, but few studies have examined the effects of nonessential amino acid supplementation in reduced-CP layer diets. Interestingly, some studies have reported that glycine supplementation improves fat digestion and FCR in laying hens (Akinde and Etop, 2014), and glutamic acid is required for bone mineralization and eggshell calcification (Pereira et al., 2019).

Most layer studies have estimated the requirement of one amino acid at a time without considering the interactive effects of other dietary amino acids. It has been demonstrated that dietary amino acids compete for intestinal uptakes via an array of amino acid transporter systems, and some amino acids have mutual antagonistic effects (Szmelcman and Guggenheim, 1966; Sheerin and Bird, 1972). Moreover, interactive effects of dietary amino acids on feed intakes in layers have been reported (Novak et al., 2004). Therefore, it is important to design amino acid requirement assays for layers that take amino acid interactions into consideration.

On the other hand, CP levels of experimental diets and their ratio to energy density could be a factor that alters amino acids requirement in laying hens because of the changes in amino acid balance and liver functions (Rama Rao et al., 2011). Moreover, performance parameters used to estimate the amino acid requirements are modified by dietary crude protein and energy levels, and amino acid requirements may not be determined with precision. Furthermore, studies in the literature are mainly over a short time period in the production

cycle which is not totally representative. Egg production is not stable throughout the production cycle; therefore, different protein and energy levels are required over time.

As mentioned previously, amino acid requirements of laying hens alter because of the numerous experimental conditions including breed, age, housing system, and basal diet. This is highlighted in variations of lysine recommendations by different breeder companies, thus lysine used as the reference amino acid (Table 3). Therefore, inconsistency of amino acid requirements that appears in the literature may not apply to the commercial level. As a solution, this review suggests that ideal amino acid ratios specific to aforementioned variables should be developed. Moreover, valuation of amino acid recommendations highly be subject to the production parameters that use optimization (Kakhki et al., 2016a). Similarly, this review observed variations of amino acid recommendations for different production parameters (weight gain, egg weight, FCR, egg production, and egg mass) in the same study for example Bregendahl et al. (2008). Therefore, it is important to choose the most appropriate parameter or combination of parameters that represent the business model of the producer. This review assumes FCR and egg mass fitting to reflect the required output from layer hens to the industry. On this mind, the suggested recommendations for lysine, sulphur-containing amino acids, threonine, tryptophan, and BCAA are tabulated in Table 16 with comparison to NRC (1994), and simple means of amino acid recommendations separated based on the breed from more recent data are tabulated in Table 17.

The average period of the first egg laying cycle is from 20 to 72 wk in commercial laying hens, and maximum egg production is obtained around 40 to 48 wk of age. Thereafter, egg production gradually decreases with increases in egg weight (Schutte et al., 1994) and reductions in eggshell quality (Roberts, 2004). Therefore, dietary strategies are required for the maintenance of egg production after the peak in the egg laying cycle. Moreover, more comprehensive data in literature are available till the end of peak production. In the future, it is more important to complete experiments to determine the ideal amino acid ratios that are required for persistent egg production rather than optimum production. However, amino acid requirements for persistent egg production may be higher than those for normal egg production, and the challenge is to provide high-density amino acid diets to older hens because aging reduces the efficiency of liver function.

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## DISCLOSURES

The authors declare that they have no financial and personal relationships with other people or organizations that can inappropriately influence their work and that 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 article.

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