Effect of dietary digestible lysine concentration on performance, egg quality, and blood metabolites in laying hens

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Primary Audience: Nutritionists, Researchers, Flock Supervisors, Quality Assurance Personnel

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SUMMARY

An experiment was conducted to evaluate the effect of dietary digestible lysine concentration on productive performance, egg quality, and blood metabolites in laying hens. A completely randomized block design was performed with 5 treatments and 6 replicates of 8 Hy-line W-36 hens each, from 32 to 44 wk of age. The treatments were 5 digestible lysine concentrations (0.657, 0.707, 0.757, 0.807, and 0.857% of diet). Feed intake was significantly increased with each increment in dietary digestible lysine concentration. Significant improvement in egg production, egg weight, egg mass, Haugh unit, and FCR were observed by an increase in lysine intake. Dietary lysine concentration did not have a significant impact on percentage of egg components, specific gravity, eggshell thickness, DM, and protein constituents of eggs. Dietary digestible lysine concentration did not have a significant effect on triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid, and total protein in plasma. The digestible lysine requirements for optimal egg production, egg mass, and egg weight (32 to 44 wk of age), based on the linear broken-line regression analysis, were 814, 810 and 778 (mg/b/d), respectively. Whereas, these values were 4, 3.9, and 6 percent less than those estimated by the quadratic broken-line model, respectively.

Key words: digestible lysine, laying hens, egg production, egg quality, blood metabolites

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DESCRIPTION OF PROBLEM

Recent advances in genetics, management, health, and behavioral science of animals provide a better feed efficiency, heavier egg, and longer peak of egg production in laying hens [1]. Reduction in nutrient excretion may lead to improved production and feed efficiency. Furthermore, strategies that reduce nutrient excretion are paramount to environmental protection goals [2]. Efficient utilization of protein in the diet depends on amount, composition, and digestibility of dietary amino acids [3], and protein utilization is more effective if the dietary amino acids profile matches the animal's requirements [4]. Feed formulation based on digestible amino acids not only reduces feed cost and supplies true requirements of birds, but also reduces environmental pollution due to lower nitrogen excretion [3]. The increase in feed costs and growing concerns of adverse effects to the environment due to nitrogen excretion through intensive poultry

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industries led nutritionists to re-evaluate protein and amino acids in poultry diets [5].

There are contradictory results in estimation of lysine requirements for laying hens. Weerden and Schutte [6] and Nathanael and Sell [7] assessed total lysine requirement by a polynomial equation to be 860 and 700 (mg/b/d), respectively. In contrast, total lysine requirement reported by Al-Bustany and Elwinger [8] was at 820 (mg/b/d) using a polynomial equation, and NRC 1994 [9] lists a lysine requirement of 690 (mg/b/d) for white egg laying hens. This difference in the estimation of lysine requirements, may result from various environmental conditions, genetic lines, basal diets, feed intakes, energy levels, several feed ingredients, nest spaces, different ages of hens [5], and the models used to estimate requirements. Some striking observations revealed that dietary protein and individual amino acids consumption may directly modify DM and protein constituents of eggs. However, the efficacy of amino acids in egg protein content manipulation has been scarcely studied [10–12]. Generally, an estimation of amino acid requirement depends on the production parameter taken into consideration for optimization [13]. The aim of this experiment was to study the effect of dietary digestible lysine concentration on productive performance, egg quality, and some blood metabolites in laying hens during 32 to 44 wk of age.

MATERIALS AND METHODS

Birds and Management

This experiment was conducted according to the comprehensive guide of animal welfare adopted at the Ferdowsi University of Mashhad, (Mashhad, Iran). Two-hundred-forty laying hens, Hy-Line W-36 with 85% of egg production were used during 32 to 44 wk of age. The experiment was designed as a complete randomized block design, with 5 treatments and 6 replicates of 8 birds each. The blocks were used in order to prevent impacts of different locations of cages. Every block contained one replicate cage for each treatment. All replicate cages were located in an upper row with the exception of 5 replicate cages in the lower row of a California cage system design. Each cage of 40 × 40 × 39 cm (L × W × H) had 4 hens. Feed and water were provided ad libitum. The house temperature ranged from 16 to 18°C and the lighting program was set at 16L: 8D throughout the experiment. Hens were weighed individually at the beginning and end of the trial.

Diets and Treatments

The diets were formulated to meet or exceed “Hy-line W-36” recommendations [14] for peak egg production, except for digestible lysine. The mash diets contained 5 concentrations of digestible lysine including 0.657, 0.707, 0.757, 0.807, and 0.857% (Table 1). The diets were provided in a way that a batch of basal diet (lowest digestible lysine concentration) was made and then divided into 5 equal portions; the lysine-HCL was added on top of each portion and mixed to make the 5 dietary treatments. Protein and digestible amino acids of the feedstuffs were analyzed with the use of NIR by Evonik Degussa Co. The analyzed concentration of crude protein and total and digestible amino acids in the basal diet is shown in Table 2. Experimental periods consisted of a 2-week equilibration phase and 12 wk of data collection that were divided into 3 consecutive periods of 28 d each.

Egg Production and Quality Traits

Egg production (egg mass and hen day) was recorded daily by registering numbers and weights of eggs produced by each replicate cage. Feed consumption was calculated as the difference between the total amount of feed issued during the 28 d period and the unconsumed residual at the end of the period. Three eggs were randomly collected from each experimental unit, in the last 3 consecutive d of each period in order to measure egg quality. Maximum width and length were measured by passing the width or length of eggs through the digital Caliper (0.01 mm) [15] to find the maximum points and then calculating the egg shape index by the following formula [16]:

$$[(\text{Egg width (mm)} / \text{egg length (mm)}) \times 100] .$$

Table 1. Ingredients and nutrients composition of basal diet.[1]

Ingredient (%)	Basal diet
Corn (CP = 7.4%)	61.16
Soybean meal (CP = 44.7%)	19.84
Corn gluten meal (CP = 54.1%)	3.50
$CaCo_3$	9.82
Vegetable oil	2.67
Dicalcium phosphate	1.91
NaCl	0.39
Vitamin Premix[2]	0.25
Mineral Premix[3]	0.25
DL-Met	0.16
L-Threonine	0.04
Calculated analysis	
ME(kcal/kg)	2900
CP (%)	15.50
Ca (%)	4.20
Available P (%)	0.48
Na (%)	0.18
Digestible Met (%)	0.37
Digestible Met+Cys (%)	0.63
Digestible Lys (%)	0.65
Digestible threonine (%)	0.53

[1] The diets were provided in a way that a batch of basal diet (lowest digestible lysine concentration) was made and then divided into 5 equal portions; the lysine-HCL was added on top of each portion and mixed to make the 5 dietary treatments.

[2] Provided in kg of diet: vitamin A (retinol), 8,800 IU; vitamin D3 (cholecalciferol), 3,300 IU; vitamin E (DL-α-tocopheryl acetate), 18.5 IU; vitamin K3 (menadione), 2.2 mg; vitamin B1 (thiamin), 2.2 mg; vitamin B2 (riboflavin), 5.5 mg; vitamin B3 (niacin), 28.0 mg; vitamin B5 (pantothenic acid), 6.6 mg; vitamin B6 (pyridoxine), 3.5 mg; vitamin B9 (folic acid), 0.7 mg; vitamin B12 (cyanocobalamin), 0.02 mg; vitamin H2 (biotin), 0.05 mg; antioxidant 1.0 mg.

[3] Provided (mg/kg of diet): Mn (manganese sulfate) 80.0, Fe (iron sulfate) 75.0, Zn (zinc sulfate) 64.0, Cu (copper sulfate) 6.0, Se (Sodium Selenite) 0.3.

After weighing individual eggs by a digital electronic scale (0.001-g) [17], the egg components, including yolk and albumen, were separated by a commercially hand-held egg separator. Paper napkins were used to eliminate the adhering of albumen residues from yolks, and then the yolks were weighed. The eggshells were washed by water, dried for 48 h, and weighed. Eggshell thickness was measured using a micrometer apparatus (0.001-mm) [18] at 3 disparate sites (top, middle, and bottom), which were averaged to calculate overall eggshell thickness. The albumen weight was calculated by subtraction of yolk and shell weights from the whole egg weight [12]. Haugh unit was calculated based on the following formula [19]:

$$\text{Haugh units} = 100 \times \log[\text{albumen height (mm)} + 7.57 - (1.7 \times \text{egg weight } (g))^{0.037}].$$

These qualitative measurements took place in less than 6 h after the egg collection. Two eggs were randomly collected from each of the replicate hens to measure DM and egg protein in the last d of the experiment. The egg yolk and albumen were attentively separated by a commercial egg separator and, after mixing and homogenization of each portion, 5 to 6 g of each sample were placed in aluminum dishes and dried in an oven at a temperature of 105°C degrees for 24 h. Samples were removed from the oven, placed in a desiccator, allowed to cool, and then were weighed [20]. Crude protein was analyzed using the SD-Kjeldahl Method [21].

Table 2. Analyzed concentration of crude protein and amino acids[1] (% of basal diet).

Items	Total	Digestible
Crude protein	15.42	–
Methionine	0.425	0.403
Cystine	0.268	0.223
Methionine + Cystine[2]	0.693	0.630
Lysine	0.733	0.657
Threonine	0.616	0.526
Tryptophan	0.166	0.143
Arginine	0.918	0.849
Isoleucine	0.623	0.561
Leucine	1.482	1.351
Valine	0.732	0.650
Histidine	0.417	0.386
Phenylalanine	0.773	0.697

[1] Measured by NIR analysis per Evonik.

[2] Methionine + Cystine estimated with separate calibration equation.

Blood Collection and Analysis

Two birds from each replicate were selected and 2.5 mL blood samples were taken from a brachial vein by 5 mL sterile syringe after 10 h feed withdrawal (8 h in dark period and 2 h in light period) [22] to assay blood components at the termination of the experiment. Plasma samples were separated and analyzed by a multi-test automatic random-access system auto analyzer [23] to measure the concentration of uric acid, protein, low-density lipoprotein (**LDL**), high-density lipoprotein (**HDL**), triglycerides, and cholesterol.

Statistical Analysis

All data were analyzed for normality using SAS software [24] through Univariate plot normal procedure. Then, data were analyzed using the General Linear Model of SAS [24]. The effect of block was not significant for any of the parameters in each or the whole experimental period. Means were separated using Tukey's test. The digestible lysine requirement intakes (mg/b/d) for optimal egg production parameters were determined by utilization of analyzed digestible lysine intakes using NLIN procedure, through linear and quadratic broken-lines regression analysis fit models as described by Robbins et al. [25].

RESULTS AND DISCUSSION

Hen Performance

The effect of digestible lysine concentration on feed consumption and nutrient intake are shown in Table 3. An increase in the dietary digestible lysine concentration had a significant impact on feed intake during the first and the whole experimental periods, and subsequently discernible differences were observed in nutrient intake. An increase in the dietary digestible lysine concentration from 0.657 to 0.757% significantly increased feed consumption from 87.4 to 95.8 (g/b/d) during the first period ($P = 0.0011$). Similarly, feed intake was increased from 93.1 to 101.1 (g/b/d) with an increase in dietary digestible lysine concentration from 0.657 to 0.757% during the whole experimental period ($P = 0.004$). However, during the second and third periods, feed intake was not altered by the dietary lysine concentration. Therefore, no significant differences were observed in any nutrient intake during these 2 periods with the exception of lysine. The digestible lysine intake, based on the feed consumption and amino acids analysis for birds fed diets containing 0.657,

Table 3. Effect of dietary lysine concentration on nutrient intake of laying hens during period 1 (32 to 36 wk), 2 (36 to 40 wk), 3 (40 to 44 wk), and the whole experiment (overall; 32 to 44 wk).[1]

Analyzed dietary digestible lysine concentration (%)	Feed consumption (g/b/d)				ME intake (kcal/b/d)				Analyzed CP intake (g/b/d)			
	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall
0.657	87.4^{c}	94.2	97.8	93.1^{b}	253^{c}	273	284	270^{c}	13.48^{c}	14.53	15.08	14.36^{c}
0.707	92.2^{b}	95.4	99.3	95.6^{b}	267^{b}	277	288	277^{c}	14.22^{b}	14.71	15.31	$14.74^{b,c}$
0.757	95.8^{a}	102.8	104.6	101.1^{a}	278^{b}	298	303	$293^{a,b}$	14.77^{b}	15.85	16.13	$15.59^{a,b}$
0.807	97.8^{a}	103.2	105.6	102.2^{a}	284^{a}	299	306	296^{a}	15.08^{a}	15.91	16.28	15.76^{a}
0.857	$94.6^{a,b}$	103.1	104.9	100.9^{a}	274^{b}	299	304	293^{b}	$14.59^{a,b}$	15.90	16.18	$15.56^{a,b}$
SEM	0.944	1.118	1.233	0.998	2.730	3.450	3.401	3.198	0.145	0.186	0.199	0.186
P-values	0.001	0.094	0.075	0.004	0.002	0.084	0.082	0.005	0.001	0.091	0.070	0.006

[a–c]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

[1] Data are means of 6 replications of 8 hens each.

0.707, 0.757, 0.807, and 0.857% digestible lysine were 612, 676, 765, 825, and 865 (mg/b/d), respectively, throughout the whole experimental period.

There were conflicting results regarding the effect of dietary lysine concentration on feed consumption. Silva et al. [2] and Al Bustany and Elwinger [8] reported significant effects of dietary supplementation of lysine on feed consumption. In contrast, the results of Nathanael and Sell [7], Schutte and Smink [4], Novak et al. [13], and Prochaska et al. [10] revealed that feed consumption was not affected by supplemental lysine.

Egg production was significantly increased with the addition of digestible lysine to the diet during each period, and throughout the experiment (Table 4). An increase in digestible lysine intake from 574 to 725 (mg/b/d) improved egg production from 80.64 to 88.08% during the first period ($P = 0.0001$). The same discernible improvement was observed during the second, third, and the whole experimental periods. Egg production was improved from 84.72 to 91.88% and 84.74 to 88.16% in response to each increment of lysine intake from 619 to 833 and 643 to 792 (mg/b/d) during the second and third periods, respectively ($P < 0.01$). An increase in digestible lysine intake from 612 to 825 (mg/b/d) significantly improved egg production from 83.37 to 91.03% throughout the whole experimental period ($P = 0.0004$). The increase in lysine intake significantly enhanced egg production in some reports [2, 7, 8, 26, 27]. Although in some other studies, egg production was not affected by a wide variation of lysine consumption [4, 10, 13].

The digestible lysine requirements for the optimum egg production during the first, second, third, and the whole experimental periods were estimated by linear broken-line fit model in the amounts of 749, 831, 852, and 814 (mg/b/d), respectively, whereas the quadratic broken-line fit model estimated higher digestible lysine requirements as 819, 841, 890, and 848 (mg/b/d) to optimize egg production during the first, second, third, and the whole experimental periods, respectively.

Applegate et al. [28] reported the total lysine requirement of 804 (mg/b/d) to optimize egg production, based on the greatest egg

Table 4. Effect of dietary digestible lysine concentration on productive performance of laying hens during periods of 1 (32 to 36 wk), 2 (36 to 40 wk), 3 (40 to 44 wk), and the whole experiment (overall; 32 to 44 wk).[1]

	Analyzed digestible lysine Intake (mg/b/d)				Egg production (%)				Egg mass (g/b/d)				FCR[2] (feed/ egg mass)			
Analyzed dietary digestible lysine concentration (%)	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall
0.657	574^{d}	619^{d}	643^{d}	612^{e}	80.64^{c}	84.72^{b}	84.74^{b}	83.37^{b}	48.86^{c}	49.22^{c}	49.99^{b}	49.36^{b}	1.79^{a}	1.91^{a}	1.96^{a}	1.89^{a}
0.707	652^{c}	674^{d}	702^{d}	676^{d}	$84.56^{b,c}$	86.74^{b}	86.62^{b}	85.97^{b}	51.80^{b}	51.65^{c}	52.57^{b}	52.01^{b}	1.78^{a}	$1.85^{a,b}$	$1.89^{a,b}$	1.84^{b}
0.757	725^{b}	$778^{b,c}$	$792^{b,c}$	765^{c}	88.08^{a}	$87.66^{a,b}$	88.16^{a}	$87.97^{a,b}$	54.14^{a}	53.39^{b}	54.56^{a}	$54.03^{a,b}$	1.77^{b}	1.93^{b}	1.92^{b}	1.87^{b}
0.807	789^{a}	833^{b}	852^{b}	825^{b}	90.42^{a}	91.88^{a}	90.80^{a}	91.03^{a}	55.70^{a}	56.88^{a}	56.83^{a}	56.47^{a}	1.76^{c}	1.81^{c}	1.86^{b}	1.81^{c}
0.857	811^{a}	884^{a}	899^{a}	865^{a}	$88.28^{a,b}$	86.46^{b}	$89.90^{a,b}$	$88.21^{a,b}$	54.32^{b}	52.95^{b}	55.14^{a}	54.13^{a}	1.74^{c}	1.95^{b}	1.90^{b}	1.86^{b}
SEM	8.360	9.049	9.186	8.881	0.880	0.866	0.887	0.877	0.543	0.538	0.552	0.544	0.015	0.017	0.018	0.018
P-values	<0.001	<0.001	<0.001	<0.001	<0.001	0.010	0.037	0.010	<0.001	0.025	0.042	0.002	<0.001	0.034	0.003	<0.001
Estimated requirement (mg/b/d)																
Linear broken-line					749	831	852	814	747	830	832	810	–	–	–	–
Quadratic broken-line					819	841	890	848	810	845	877	843	–	–	–	–

[a–e] Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

[1] Data are means of 6 replications of 8 hens each.

[2] The digestible lysine intakes did not fit the linear and quadratic broken-line models to estimate the optimum FCR in each and the whole experimental period.

production rate of 89.06% in Hy-line W-36 layers during 33 to 44 wk of age. Their estimation was close to the results of our study at 814 and 848 (mg/b/d), based on the prediction by linear and quadratic broken-line regressions analysis for 87.03% average egg production. Nathanael and Sell [7] also determined the total lysine requirement by a polynomial equation to be 702 (mg/b/d) in White Leghorn hens with 80% average egg production during 42 to 54 wk of age.

Egg mass was significantly enhanced by increasing lysine intake during all experimental periods (Table 4). Egg mass was improved from 48.86 to 54.14 (g/b/d) with an increase in digestible lysine intake from 574 to 725 (mg/b/d) during the first period ($P = 0.0001$). An increment in digestible lysine consumption from 619 to 833 (mg/b/d), increased egg mass from 49.22 to 56.88 (g/b/d) during the second period ($P = 0.0004$), whereas the egg mass was improved from 49.99 to 54.56 (g/b/d) with an increase in digestible lysine intake from 643 to 792 (mg/b/d) during the third period ($P = 0.0011$). In addition, increasing digestible lysine intake from 612 to 825 (mg/b/d) significantly improved egg mass from 49.36 to 56.47 (g/b/d) throughout the whole experiment ($P = 0.0040$). The digestible lysine requirements for the optimal egg mass estimated by the linear broken-line model were 747, 830, 832, and 810 (mg/b/d) during the first, second, third, and the whole experimental periods, respectively. However, the quadratic broken line model estimated the requirements at 810, 845, 877, and 843 (mg/b/d) during the first, second, third, and the whole experimental periods, respectively. In numerous studies, a conspicuous significant improvement in egg mass was observed by augmentation of supplemental lysine [2, 4, 8, 28].

Applegate et al. [28] reported a total lysine requirement of 804 (mg/b/d) to obtain optimal egg mass (55.44 g/b/d) in Hy-line W-36 hens during 33 to 44 wk of age. Their estimation of total lysine requirement was almost similar to that of digestible lysine estimated by the linear broken-line model (804 vs. 810 mg/b/d), but 4.6% lower than that estimated by the quadratic broken-line analysis model in our study to obtain an egg mass of 55.30 and 55.11 (g/b/d), respectively. In contrast, Bregendahl et al. [29] predicted the total lysine requirements by the single-slope broken-line model to be 508 and 538 (mg/b/d) to maximize egg mass of 53.3 and 49.4 (g/b/d) in Hy-line W-36 hens during 28 to 34 and 52 to 58 wk of age, respectively.

The FCR was significantly different ($P < 0.01$) among the treatments during each period and throughout the experiment (Table 4). Feed conversion ratio was decreased from 1.79 to 1.76 by an increase in digestible lysine intake from 574 to 789 (mg/b/d) during the first period. The FCR decreased from 1.91 to 1.81 (g/b/d) as digestible lysine consumption increased from 619 to 833 (mg/b/d) during the second period, and it decreased from 1.96 to 1.92 (g/b/d) as digestible lysine intake increased from 643 to 792 (mg/b/d) during the third period. In addition, a 4.4% reduction in FCR (1.89 vs. 1.81 g/b/d) during the whole experimental period was observed as digestible lysine intake increased from 612 to 825 (mg/b/d). The FCR responses to digestible lysine intake of birds did not fit the linear or quadratic broken-line models to predict the optimum requirements for FCR in each or the whole experimental periods. Applegate et al. [28] reported the optimum total lysine requirement of 753 (mg/b/d) was necessary to obtain the best FCR of 1.99 in Hy-line W-36 layers during 33 to 44 wk of age.

Generally, these discrepancies in production performance during our study and other ones may be due to various environmental conditions, bird strains, age, stage of egg production, diets, and also the models used for the estimations.

Body weight changes were not significantly affected by lysine intake. Similar to our results, Schutte and Smink [4] observed no significant impact on weight of layers fed diets containing different concentrations of lysine (ranging from 717 to 1021 mg/b/d) with constant energy (2810 Kcal/kg) and protein concentration (16.4% crude protein). Although egg production, egg mass, and egg weight were affected by dietary lysine concentration. Prochaska et al. [10] reported no significant effect on body weight, egg production, ME, and CP intake of layers consuming 677 to 1613 (mg/b/d) lysine, but egg weight significantly improved as lysine intake increased.

Egg Quality

The effect of dietary digestible lysine concentration on egg weight and the percentage of albumen, yolk, and shell are shown in Table 5. An increment of digestible lysine intake significantly affected egg weight during the second, third, and the whole experimental periods ($P < 0.05$). Increasing digestible lysine consumption from 619 to 833 and 643 to 852 (mg/b/d) significantly enhanced egg weight from 58.10 to 61.91 and 58.99 to 62.59 g in the second and third periods, respectively. Increasing digestible lysine consumption from 612 to 765 (mg/b/d) significantly enhanced average egg weight from 59.23 to 61.42 (g) throughout the experiment. Digestible lysine requirements were estimated by the linear broken-line fit model to be 811, 745, and 778 (mg/b/d), and by the quadratic broken-line fit model to be 855, 814, and 828 (mg/b/d) to achieve optimum egg weights during the second, third, and the whole experimental periods, respectively. The digestible lysine intake did not fit the linear and quadratic broken-line models to estimate the optimum egg weight in the first period.

The results were in agreement with findings of Applegate et al. [28], Bregendahl et al. [29], Neto et al. [27], Silva et al. [2], Nathanael and Sell [7], Schutte and Smink [4], Al Bustany and Elwinger [8], Novak et al. [13], and Prochaska et al. [10], who reported a significant improvement in egg weight with dietary lysine supplementation. Bregendahl et al. [29] estimated the total lysine requirement by the single-slope broken-line model to be 649 and 573 (mg/b/d) to maximize egg weight of 53.3 and 61.1 g in Hy-line W-36 layers during 28 to 34 and 52 to 58 wk of age, respectively. Also Nathanael and Sell [7] used a polynomial equation and estimated total lysine requirement of 700 (mg/b/d) for optimal egg weight of 56.87 g in Hy-line W-36 layers during 22 to 42 wk of age.

Albumen, yolk, and eggshell percentages of eggs were not affected by lysine supplementation during each period and throughout the experiment (Table 5). Also, Applegate et al. [28] reported that the increase in lysine intake as well as Met, Thr, and Ile did not significantly affect egg component percentages. In contrast, Novak et al. [13] reported a significant effect

Table 5. Effect of dietary digestible lysine concentration on egg qualitative traits of laying hens during periods of 1 (32 to 36 wk), 2 (36 to 40 wk), 3 (40 to 44 wk), and the whole experiment (overall; 32 to 44 wk).[1]

Analyzed dietary digestible	Egg weight (g)[2]				Albumen (%)				Yolk (%)				Shell (%)			
lysine concentration (%)	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall
0.657	60.59	58.10^{b}	58.99^{c}	59.23^{b}	64.74	63.87	64.07	64.23	24.98	26.69	26.66	26.10	10.28	9.44	9.30	9.67
0.707	61.26	59.55^{a,b}	60.69^{b,c}	60.50^{a,b}	65.58	63.89	63.29	64.25	24.96	26.53	27.14	26.21	9.46	9.58	9.57	9.54
0.757	61.47	60.91^{a,b}	61.89^{a,b}	61.42^{a}	64.23	64.20	63.08	63.84	25.93	26.03	27.53	26.50	9.84	9.77	9.39	9.67
0.807	61.60	61.91^{a}	62.59^{a}	62.03^{a}	65.79	63.74	63.48	64.34	24.68	26.60	27.10	26.13	9.53	9.66	9.42	9.54
0.857	61.53	61.24^{a,b}	61.33^{b}	61.37^{a,b}	66.04	63.83	62.81	64.23	24.69	26.79	27.82	26.44	9.27	9.38	9.37	9.34
SEM	0.599	0.603	0.604	0.602	0.641	0.619	0.615	0.624	0.240	0.258	0.283	0.254	0.130	0.152	0.093	0.125
P-values	0.061	0.045	0.037	0.040	0.284	0.967	0.686	0.721	0.419	0.744	0.550	0.600	0.181	0.384	0.930	0.473
Estimated requirement (mg/b/d)																
Linear broken-line	–	811	745	778	–	–	–	–	–	–	–	–	–	–	–	–
Quadratic broken-line	–	855	814	828	–	–	–	–	–	–	–	–	–	–	–	–

$^{a–c}$Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

[1] Data are means of 6 replications of 9 eggs each.

[2] The digestible lysine intake did not fit the linear and quadratic broken-line models to estimate the optimum egg weight in the first period.

on percentage of albumen and yolk in response to supplemental lysine in DeKalb Delta layers during 20 to 63 wk of age. In the current study, the av erage digestible lysine intake was 748 (mg/b/d), but the average total lysine intake in the experiment of Novak et al. [13] was 909 (mg/b/d). Prochaska et al. [10] performed 2 distinct experiments with different dietary lysine concentrations and age of hens and reported different results. In the first experiment, the percentages of yolk and albumen were not affected when the total lysine intake changed from 638 to 1165 (mg/b/d) in Hy-line W-36 layers. However, the percentages of yolk and albumen were significantly affected when lysine intake changed from 677 to 1613 (mg/b/d) during 42 to 64 wk of age in the second experiment. An explanation for these inconsistent results may be due to differences in the intake of lysine and other nutrients.

The effect of digestible lysine consumption on egg shape index, Haugh unit, specific gravity, and eggshell thickness are shown in Table 6. The Haugh unit was significantly affected by digestible lysine intake during each and the whole experimental period ($P < 0.01$). An increasing digestible lysine intake from 574 to 789 (mg/b/d) increased the Haugh unit from 86.59 to 92.43 during the first period. Whereas, an increase in digestible lysine intake from 619 to 833 (mg/b/d) increased the Haugh unit from 85.07 to 90.85 in the second period. The Haugh unit was significantly improved from 83.24 to 89.32 when digestible lysine intake increased from 643 to 852 (mg/b/d) during the third period. An increase in digestible lysine consumption from 612 to 825 (mg/b/d) resulted in a significant augmentation of the Haugh unit from 84.97 to 90.87 in the whole experimental period.

Figueiredo et al. [30] found that the Haugh unit was quadratically related to an increment of dietary digestible lysine concentration ranging from 0.542 to 0.879% in Hy-line W36 layers during 42 to 58 wk of age. They claimed that a high concentration of digestible lysine in a diet that contains adequate CP concentration could be the factor that deteriorates albumen quality due to an amino acid imbalance profile in the diet.

The Haugh unit was quadratically related to lysine intake during the second, third, and the

Table 6. Effect of dietary digestible lysine concentration on egg qualitative traits of laying hens during periods of 1 (32 to 36 wk), 2 (36 to 40 wk), 3 (40 to 44 wk), and the whole experiment (overall; 32 to 44 wk).[1]

	Egg shape index (%)				Haugh unit[2]				Specific gravity (g/cm3)				Shell thickness (mm)			
Analyzed dietary digestible lysine concentration (%)	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall	Period 1	Period 2	Period 3	Overall
0.657	76.81	76.96	77.73	77.17	86.59^{c}	85.07^{b}	83.24^{b}	84.97^{c}	1.083	1.085	1.077	1.082	0.377	0.390	0.370	0.379
0.707	77.61	79.24	77.75	78.20	88.70^{b,c}	86.51^{a,b}	85.93^{a,b}	87.05^{b}	1.084	1.084	1.080	1.083	0.371	0.388	0.372	0.377
0.757	76.30	77.32	79.10	77.57	90.26^{a,b}	87.79^{a,b}	87.23^{a,b}	88.43^{b}	1.081	1.084	1.080	1.082	0.384	0.392	0.378	0.385
0.807	76.80	77.61	75.87	76.76	92.43^{a}	90.85^{a}	89.32^{a}	90.87^{a}	1.083	1.082	1.079	1.081	0.377	0.380	0.373	0.377
0.857	76.43	77.46	76.01	76.63	92.57^{a}	89.51^{a}	88.76^{a}	90.28^{a,b}	1.083	1.080	1.078	1.080	0.376	0.398	0.368	0.381
SEM	0.743	0.753	0.734	0.742	0.977	0.858	0.850	0.861	0.010	0.011	0.010	0.011	0.013	0.010	0.007	0.004
P-values	0.927	0.348	0.141	0.089	<0.001	0.007	0.004	<0.001	0.842	0.361	0.645	0.396	0.979	0.825	0.903	0.603
Estimated requirement (mg/b/d)																
Linear broken-line	–	–	–	–	–	831	851	824	–	–	–	–	–	–	–	–
Quadratic broken-line	–	–	–	–	–	849	893	835	–	–	–	–	–	–	–	–

$^{a–c}$Values with uncommon superscripts within each column are significantly different ($p < 0.05$).

[1] Data are means of 6 replications of 9 eggs each.

[2] The digestible lysine intake did not fit the linear and quadratic broken-line models to estimate the optimum Haugh unit in the first period (32 to 36 wk).

Table 7. Effect of digestible lysine intake on egg DM and protein content of laying hens determined at the end of the experiment.[1]

	Yolk		Albumen		Total egg (without shell)	
Analyzed digestible lysine intake (mg/b/d)	DM (%)	Protein[2]	DM (%)	Protein[2]	DM (%)	Protein[2]
612	51.19	16.65	11.62	10.35	20.82	10.99
676	51.09	16.92	11.71	10.88	20.91	11.43
765	51.28	16.99	12.50	11.10	21.57	11.59
825	51.34	17.12	12.34	11.41	21.35	11.81
865	51.49	17.33	12.41	11.55	21.58	12.00
SEM	0.500	0.255	0.143	0.150	0.238	0.138
***P*-values**	0.128	0.381	0.198	0.165	0.121	0.089

[1] Data are means of 6 replications of 2 eggs each at 44 wk of age.

[2] Protein in 100 g of egg DM.

Table 8. Effect of digestible lysine intake on blood metabolites of laying hens determined at the end of the experiment.[1]

Analyzed digestible lysine intake (mg/b/d)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Uric acid (mg/dL)	Total Protein (g/dL)
612	101	105	59.01	40.32	5.20	5.93
676	100	108	58.13	42.20	5.35	6.05
765	102	102	57.85	41.29	5.31	6.54
825	103	107	57.29	41.52	5.35	6.31
865	101	108	58.14	43.10	5.60	6.49
SEM	0.980	1.085	0.560	0.958	0.458	0.485
***P*-values**	0.358	0.259	0.315	0.570	0.191	0.346

[1] Data are means of 6 replications of 2 blood samples, each at 44 wk of age.

whole experimental periods. The estimated digestible lysine requirements by linear broken-line regression analysis were 831, 851, and 824 (mg/b/d) to achieve an optimal Haugh unit during the second, third, and the whole experimental periods, respectively. In contrast, the quadratic broken-line regression analysis model estimated the digestible lysine intake to be 849, 893, and 835 (mg/b/d) to optimize the Haugh unit in the second, third, and the whole experimental periods, respectively. Egg shape index, specific gravity, and eggshell thickness were not affected by lysine supplementation during each and the whole experimental period.

Dry Matter and Protein Contents of Egg

Dry Matter and protein contents of yolk, albumen, and the whole egg liquid were not influenced by digestible lysine intake in the whole experimental period (Table 7). These observations were in agreement with Applegate et al. [28] but they are inconsistent with the observations of Prochaska et al. [10] and Novak et al. [13]. In the experiment of Novak et al. [13], a significant effect on protein contents of yolk and albumen was observed with 909 (mg/b/d) lysine intake, but no significant effect on DM of yolk, albumen, and the whole egg. Prochaska et al. [10] conducted 2 trials and revealed significant changes in DM and protein contents of both yolk and albumen when Hy-line W-36 layers lysine intake was 638, 828, 1,062, and 1,165 (mg/b/d), respectively during 23 to 38 wk of age. Whereas, DM and protein contents of only albumen significantly changed with a lysine intake of 677, 1,154 and 1,613 (mg/b/d), respectively, during 42 to 64 wk of age in the second trial. The main reason for this inconsistency may be due to a discrepancy in lysine intake and age of birds. Modification of DM and protein contents of eggs is expected to occur by over-consumption of lysine relative to the real requirement of birds. Apparently, by increasing dietary lysine concentration relative to its competitors of absorption (i.e., Arg and His), a greater proportion of

Table 9. Summary of requirements of digestible lysine intake for optimization of egg production parameters of laying hens estimated by linear and quadratic broken-line regression fit models.[1]

	Linear broken-line regression analysis						Quadratic broken-line regression analysis					
Models Parameter	Estimated digestible lysine requirement[2]	Lower 95% confidence Levels	Upper 95% confidence Levels	Predicted value[3]	R^2	R^2 adj	Estimated digestible lysine requirement[2]	Lower 95% confidence Levels	Upper 95% confidence Levels	Predicted value[3]	R^2	R^2 adj
Egg production												
32 to 36 wk	749	694	804	89.35	0.96	0.92	819	654	983	89.30	0.94	0.87
36 to 40 wk	831	682	980	89.03	0.84	0.78	841	696	986	88.82	0.82	0.76
40 to 44 wk	852	776	928	90.14	0.97	0.96	890	681	1098	89.60	0.92	0.92
32 to 44 wk	814	667	961	89.62	0.86	0.82	848	640	1056	89.34	0.85	0.82
Egg mass												
32 to 36 wk	747	696	798	55.01	0.97	0.96	810	682	937	54.95	0.96	0.94
36 to 40 wk	830	585	1074	54.91	0.73	0.70	845	642	1048	54.60	0.72	0.68
40 to 44 wk	832	742	924	55.90	0.93	0.91	877	698	1055	55.81	0.92	0.89
32 to 44 wk	810	686	933	55.30	0.89	0.86	843	657	1029	55.11	0.89	0.85
Egg weight[4]												
36 to 40 wk	811	741	880	61.70	0.96	0.93	855	716	993	61.50	0.963	0.94
40- to 44 wk	745	660	829	61.93	0.89	0.86	814	698	930	61.65	0.89	0.87
32 to 44 wk	778	710	846	61.70	0.94	0.92	828	697	959	61.68	0.95	0.93

[1] The digestible lysine intake did not fit the linear and quadratic broken-line models to estimate the optimum FCR in each and the whole experimental period.

[2] Expressed as mg/bird/d.

[3] Predicted values by models to obtain optimum egg production (%); optimum egg mass (g/b/d); and optimum egg weight in g.

[4] The digestible lysine intake did not fit the linear and quadratic broken-line models to estimate the optimum egg weight in the first period (32 to 36 wk).

dietary lysine is utilized. In addition, elevation of amino acid concentrations in plasma causes an increase in insulin secretion by the pancreas. Two functions of insulin are elevation in amino acid uptake and protein synthesis [10].

Blood Metabolites

None of the triglyceride, cholesterol, LDL, HDL, uric acid, or total protein in plasma was significantly affected by lysine intake (Table 8). Chi and Speers [31] observed no significant effects on uric acid in plasma with an increase in dietary lysine supplementation. There is a lack of information on the effect of lysine on blood metabolites in layer hens.

Finally, the summary of digestible lysine requirements for the optimization of egg production, egg mass, and egg weight estimated by linear and quadratic broken-line fit models are shown in Table 9. The predicted digestible lysine requirements throughout the experiment using the linear broken-line model for optimal egg production, egg mass, and egg weight were about 4.0, 3.9, and 6% lower than those predicted by the quadratic broken-line model, respectively. The linear broken-line model estimated slightly higher lysine requirements for egg production (89.62 vs. 89.34 percent), egg mass (55.30 vs. 55.11 g/b/d), and egg weight (61.70 vs. 61.68 g) than the quadratic broken-line model.

CONCLUSIONS AND APPLICATIONS

1. The digestible lysine requirements estimated by the linear broken-line fit model to optimize egg production, egg mass, egg weight, and Haugh unit were 814, 778, 810, and 824 (mg/b/d), respectively.
2. The digestible lysine requirements estimated by the quadratic broken-line model to optimize egg production, egg mass, egg weight, and Haugh unit were 848, 843, 828, and 835 (mg/b/d), respectively.
3. The digestible lysine requirements estimated by the quadratic broken-line model were greater than those estimated by the linear broken-line model, although, the pinnacle points of production parameters predicted by the quadratic broken-line model were slightly lower than those in the linear broken-line model.
4. The digestible lysine intake did not affect the percentage of albumen, yolk, DM, protein contents of eggs, or blood metabolites.

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