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Original Article

The Optimum Ratio of Dietary Digestible Valine: Lysine for Laying Hens During the Peaking Phase

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ABSTRACT

To determine the digestible valine (dVal) requirement and dVal to digestible lysine ratio (dVal: dLys) for laying hens, this experiment was performed on three hundred sixty Hy-Line W36 hens during the peak egg production phase (28 to 40 weeks of age), based on a completely randomized design with six treatments and five replicates of 12 birds each. The six dietary treatments were graded levels of dVal: dLys ratios: 0.82 (basal diet), 0.86, 0.90, 0.94, 0.98, and 1.02. The dVal: dLys ratio of 0.82 led to the lowest performance, indicating that valine is the first limiting amino acid in this ratio. The graded increase in the dietary dVal: dLys ratio improved egg weight (EW, $p<0.01$), egg mass (EM, $p<0.05$), and feed conversion ratio (FCR, $p<0.01$). The highest performance was observed at the dVal: dLys ratio of 0.94. Serum albumin, uric acid, and triglycerides were significantly ($p<0.01$) affected by the dVal: dLys ratio; the highest level of albumin, and the lowest levels of triglycerides and uric acid were observed at the ratios of 0.98, 0.98, and 0.94, respectively. The graded increase in dVal: dLys ratio improved ($p<0.05$) the egg quality traits (albumen and yolk height, Haugh unit, albumen ratio, and yolk color). Based on regression analysis, the response curves for EW and EM showed the best fit with the linear broken line (LBL) model, while the quadratic polynomial (QP) model was the best fit for FCR. In conclusion, the optimum dVal: dLys ratio was estimated at 0.93, which equals 695 mg dVal/ hen/ day.

INTRODUCTION

Laying hens have been under extensive genetic selection for decades, and their egg production, feed efficiency, and productivity have steadily increased (Elliott, 2008; Anderson *et al*., 2013). Consequently, it is reasonable to assume that their nutrient requirements have changed to match the increase in egg production. Therefore, it is necessary to periodically re-evaluate the response of laying hens to dietary nutrients, including essential amino acids, because they are the key to achieving genetic potential (Bregendahl *et al*., 2008).

When formulating diets, amino acid levels should be as close as possible to the recommended levels (Rostagno *et al*., 2017), as amino acid deficiencies can dramatically reduce performance. On the other hand, over-consumption of amino acids causes an increased metabolic load to the bird, excretion of more uric acid, extra nitrogen in the excreta, and reduction of dietary metabolizable energy (Leeson & Summers, 2005). Therefore, formulating diets based on digestible amino acids meets the real needs of the bird and leads to economic savings (Batman *et al*., 2008). Deficiency or oversupply of limiting amino acids can be prevented by formulating diets using synthetic amino acids. However, the gradual reduction of dietary protein by the use of synthetic amino acids will lead to a situation in which the other amino acids will limit the performance of birds; and these amino acids include the branched-chain amino acids, Val and Ile (Peganova & Eder, 2002). Increasing the use of synthetic amino acids requires a better understanding of the impact of dietary amino acid levels in laying hens (Liu & Selle, 2017). Considering that commercial access to synthetic Val is economically feasible (Kidd *et al*., 2013), an accurate estimate of the Val requirement is critical.

Val is the fourth limiting amino acid in poultry diets, after methionine, lysine, and threonine. It is an essential amino acid for egg protein synthesis (Fisher & Johnson, 1956; Lelis *et al*., 2014). Val, Leu, and Ile, referred to as branched-chain amino acids (BCAAs), are hydrophobic amino acids and play a crucial role in the structure of proteins (Konashi *et al*., 2000). The proper balance of BCAAs in poultry diets improves energy and protein metabolism (Han *et al*., 1992; Fernandez *et al*., 1994). In addition to protein synthesis, adequate intake of Val is essential for maintaining intestinal immunity, insulin secretion, amino acid uptake into the brain, antioxidant capacity, and a large number of physiological functions in poultry (Davis & Fiorotto, 2009; Azzam *et al*., 2015; Dong *et al*., 2016; Wen *et al*., 2019). On the other hand, BCAAs regulate the metabolism of fatty acids in the liver (Bai *et al*., 2015), which is a critical organ for egg production. Hepatic production of yolk components and lipoproteins are limiting factors in egg formation (Macelline *et al*., 2021).

Since the publication of NRC (1994), few studies have evaluated the Val requirement of high-producing laying hens. Moreover, the results of these studies are inconsistent. NRC (1994) recommended the total Val requirement of 700 mg/hen/day (Val: Lys ratio 1.01) Harms & Russell (2001) estimated the total Val requirement for maximum egg mass in Hy-Line W-36 commercial layers to be 619 mg/day at 39 to 47 weeks of age. Bregendahl *et al*. (2008) estimated the ratio of dVal: dLys to be 0.93 (501 mg per hen day) at 26 to 34 weeks of age in Hy-Line W-36 laying hens. Lelis *et al*. (2014) reported a ratio of 0.92 (567 mg per day) in the diet of Dekalb brown laying hens aged 42-54 weeks. Wen *et al*. (2019) estimated the total Val requirement of 597.3, 591.9, and 500.5 mg per day for Hy-Line W-36 laying hens aged 41 to 60 weeks for egg mass, egg production, and feed conversion ratio, respectively. The latest Hy-Line recommendation (2020) for standardized ileal digestible (SID) Val requirement during peak egg production is 704 mg/ hen/ day, and the calculated ratio of SID Val: Lys is 0.88. The Lohman guidline for LSL layers (2019) recommends dVal requirement of 700 mg/ hen/ day in the peaking phase, and the calculated ratio of dVal: dLys is 0.87. The recent edition of Brazilian tables (Rostagno *et al*., 2017) recommends a mean dVal requirement of 719 and 742 for low-standard and standard-high performing laying hens, respectively, corresponding to the dVal: dLys ratio of 0.93.

According to the above-mentioned, it is clear that few experiments have been conducted to determine the dVal requirement of high-producing laying hens, particularly during the peaking phase. Furthermore, the recommended values are variable due to strain, age, feeding strategies, rearing system, and statistical methods. Moreover, the existing literature (Bregendahl *et al*. 2008; Lelis *et al*. 2014; and Wen *et al*. 2019) has reported contradictory results regarding the impact of Val supplementation on egg quality traits. Therefore, the present study aimed to evaluate the effect of graded dVal: dLys ratios on performance, egg quality traits, and serum biochemical parameters of laying hens, and determine the dVal requirement of laying hens at the peak egg production phase.

MATERIALS AND METHODS

Birds and management

All experimental procedures were approved by the Bioethics Committee of the University of Zanjan (protocol no. ZNU- 44938- 2019). A total of 360 commercial laying hens (Hy-Line W36) were used in this experiment. The hens were housed in an environmentally controlled house (20 ± 2 °C). Birds of two adjacent cages (12 birds) were considered as an experimental unit. The production rate of the hens was nearly identical at the start of experiment (94 % hen day production at the age of 28 weeks). The mean EP during the experimental period (28 to 40 wk.) was 91.87, and the corresponding mean EP for Hy-Line W-36 hens (Hy-line management guide, 2020) is 93.48 %. During twelve weeks of the experiment (from 28 to 40 weeks of age), all hens had free access to feed and water. The photoperiod was 16 h light: 8 h dark. Egg numbers and mortality of the hens was recorded daily, egg weight (EW) was determined twice weekly, and feed intake (FI) was measured weekly. Egg mass (EM) and feed conversion ratio (FCR) were then calculated using these figures.

Experimental diets

A basal diet (BD, Table 1) based on corn-soybean meal was formulated to meet or exceed the standardized ileal digestible (SID) amino acid requirements recommended by Hy-Line (2020) except Val. The SID Val content of the BD was 0.64%, which was lower than the Hy-Line (2020) recommendation.

The dVal: dLys ratio of the BD was 0.82, and the corresponding ratio by Hy-Line (2020) was 0.88. Before BD formulation, all feed ingredients were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and calcium (AOAC, 1995). Amino acid analyses of ingredients were performed by high performance liquid chromatography (Knauer, Germany) after hydrolysis by hydrochloric acid (6 N) and pre-column derivatization by orthophtaldehyde (OPA). SID values for all amino acids were calculated by the AminoDat5© software (Evonik, Germany).

Table 1 – Ingredients composition and nutrient levels of the basal diet.

Ingredient	(%)
Corn	56.96
Corn starch	0.20
Soybean meal	23.86
Calcium carbonate	9.24
Soy oil	3.41
Wheat bran	3.00
Di-calcium phosphate	1.85
Vitamin premix[1]	0.30
Mineral premix[2]	0.30
Salt (iodized)	0.29
Sodium bicarbonate	0.13
DL-Methionine	0.25
L-Lysine HCl	0.11
L-Threonine	0.05
L-Isoleucine	0.04
L-Arginine	0.01
Nutrients (Analyzed values)	(%)
Metabolizable energy (calculated, kcal/kg)	2844
Crude protein	15.35
SID lysine	0.78
SID methionine	0.45
SID methionine+cystine	0.66
SID Threonine	0.55
SID Arginine	0.89
SID Valine	0.64
SID Leucine	1.16
SID Isoleucine	0.61
SID Tryptophan	0.16
Calcium	4.48
Avail. Phosphorus	0.49
Sodium	0.19
Potassium	0.64
Chloride	0.24
DCAD	190.0
Linoleic	2.99
CF	3.40

[1] Vitamin premix: Supplied per kg of diet: 8000 IU vit A, 3500 IU vit D3, 25 IU vit E, 3 mg vit K, 250 mg choline chloride, 6 mg riboflavin, 30 mg niacin, 10 mg calcium pantothenic., 0.5 mg folic acid, 1.5 mg thiamine, 2.5 mg pyridoxine, 150 µg biotin, 10 µg vit. B12

[2] Mineral premix: Supplied per kg of diet: 40 mg Fe, 10 mg Cu, 90 mg Zn, 0.25 mg Se, 100 mg Mn., 0.87 mg Iodine.

The 360 hens were randomly assigned to six dietary treatments comprising the BD and five graded additions of Val. Synthetic L-Val (97 % feed grade, CJ, South Korea) was supplemented to the BD in 0.03% increments, resulting in experimental diets containing 0.64 (BD), 0.67, 0.70, 0.73, 0.76 and 0.79 % SID Val, respectively. The added L-Val was substituted for corn starch in the BD. The Val to lysine ratios of the experimental diets were 0.82(BD), 0.86, 0.90, 0.94, 0.98, and 1.02. Each experimental diet was fed to 60 laying hens in a completely randomized design with 5 replications.

Sampling and blood parameters

Blood samples were taken from wing vein of the hens (two birds per replicate, and 10 birds in each treatment) at weeks 5 and 10 of the experiment. Serum concentrations of albumin, total protein, uric acid, glucose, total cholesterol, triglyceride, calcium, and phosphorus were determined colorimetrically by an auto-analyzer (Hitachi, Japan) using commercially available kits.

Egg Quality and Composition

At 3, 6, and 9 weeks of the experiment, six eggs from each replication (30 eggs per treatment) were randomly collected to assess egg quality criteria. Egg specific gravity were measured by floating the eggs in salt solutions with different densities (1.070, 1.075, 1.080, 1.085, 1.090, 1.095, and 1.100). The eggs were then broken, and thick albumen height (CENCo® spherometer, USA), and yolk color (DSM Roche fan) were measured. Yolk and albumen were then separated and weighted. Eggshell thickness was measured at the sharp end, equator, and blunt end of the egg, using a digital micrometer (Mitotoyo, Japan). Egg shell weight was determined using a digital scale (Ohaus, Germany) after drying.

Statistical Analysis

The experimental design was a completely randomized design with 6 treatments and 5 replicates. The data were subjected to analysis of variance (ANOVA) by the general linear model (GLM) procedure of SAS software (SAS Institute, 2003). Tukey test was used to compare the significant differences among treatment means. The significance level was considered at $P< 0.05$. The SID Val requirements were estimated using linear broken-line (LBL), quadratic broken-line (QBL), and quadratic polynomial (QP) regression models (Robbins *et al.*, 2006). The best model was selected based on goodness of fit criteria such as coefficient of determination (R^2) and residual sum of square (SSE).

RESULTS

The dVal: dLys ratio of 0.82 led to the lowest performance, indicating valine is the first limiting amino acid in this diet (Table 2). The graded increase in dVal: dLys ratio significantly increased egg weight (EW, $p<0.01$), egg mass (EM, $p<0.05$), and decreased (improved) the feed conversion ratio (FCR, $p<0.01$). The best performance was observed with the dVal: dLys ratio of 0.94. EW and EM showed linear increases with the increasing dVal: dLys up to the ratio of 0.94 ($p<0.01$). FCR showed a linear improvement ($p<0.01$) up to the ratio of 0.94. The dietary dVal: dLys levels had not significant effect on EP and FI.

Table 2 – Effects of dietary dVal: dLys ratios on the performance of laying hens (28 to 40 weeks of age).

	Dietary SID Val: Lys							*p*-Values		
	0.82	0.86	0.90	0.94	0.98	1.02	SEM	ANOVA	L	Q
EP	91.3	91.7	92.0	92.5	91.8	91.9	0.70	0.761	0.313	0.365
EW	58.5[c]	58.5[c]	58.9[bc]	60.0[a]	59.4[a]	59.3[ab]	0.21	0.003	0.0002	0.0308
EM	53.4[b]	53.7[b]	54.2[ab]	55.5[a]	54.6[ab]	54.5[ab]	0.46	0.031	0.0088	0.077
FI	97.3	96.5	95.3	95.1	94.1	95.3	1.33	0.600	0.131	0.372
FCR	1.821[a]	1.797[a]	1.757[b]	1.714[b]	1.716[b]	1.748[ab]	0.025	0.023	0.0035	0.059

[a-c] Means in a row with different superscripts are significantly different ($p<0.05$).

EP: egg production, EW: egg weight, EM: egg mass, FI: feed intake, FCR: feed conversion ratio, SEM: standard error of the means, L: linear, Q: quadratic.

The optimal SID Val requirement for maximum EW, EM, and minimum FCR were determined by different regression models (Table 3). The estimated SID Val requirement based on EW response by LBL model was estimated at 694.00 mg/day with a response plateau for EW at 59.512 grams. Using the QP model, the SID Val requirement was estimated at 650.75 mg/day at 95% of maximum response. By the QBL model, the SID Val requirement of EW was estimated at 717.80 mg/day at 99% of maximum response. Based on the goodness of fit criteria, the best fit was achieved by the LBL model and the estimated requirement for EW was 694.00 mg/day SID Val, and the respective ratio of SID dVal: dLys was 0.931 (Table 3).

The SID Val requirements for maximum EM were estimated at 694.00, 612.04 and 717.74 mg/day by the LBL, QP and QBL models, respectively (Table 3). The best fit criteria were obtained using the LBL model, and therefore the estimated dietary SID Val requirement based on EM was 694.00 mg/day, and the respective ratio of SID Val: Lys was 0.931. Using FCR as the response criterion, the SID Val requirements for minimum FCR by LBL, QP, and QBL models were 712.00, 736.25 and 718.74 mg/day, respectively (Table 3). The best fit was attributed to the QP model for FCR, and the SID Val requirement was estimated to be 736.25 mg/day and the respective ratio of SID Val: Lys was 0.987. On average, the estimated daily SID Val requirement was 694.81 mg/day, and the respective dVal: dLys ratio was 0.931.

Albumen height ($p<0.05$), Haugh unit ($p<0.01$), yolk color index ($p<0.01$), albumen to egg weight ratio ($p<0.05$), and yolk height ($p<0.05$) were increased by increasing dietary dVal: dLys levels (Table 4). Albumen height increased linearly by Val supplementation of the basal diet up to the ratio of 1.02. Haugh unit increased quadratically up to the ratio of 0.94 and then slightly decreased at the ratio of 1.02. Yolk color index increased linearly up to the ratio of 1.02. Albumen to egg weight ratio increased quadratically up to the ratio of 0.90, and then plateaued. Yolk height increased linearly up to the ratio of 0.98. Dietary dVal: dLys ratio did not

Table 3 – Estimated dVal requirement and SID Val: Lys ratio by different regression models for performance variables.

Variable	Model	Equations	R^2	Requirement (mg/day)	dVal: dLys ratio	Residual sum of squares (SSE)
	LBL	59.5115-0.0149(694.00-Val)	0.764	694.00	0.931	0.3774
EW	QP	$-0.0002\times Val^2+0.274\times$ Valin-36.92	0.510	650.75	0.873	0.785
	QBL	$59.48-0.0001\times(725.06-Val)^2$	0.645	717.80	0.962	0.5701
	LBL	$54.977-0.0213\times(694.00-Val)$	0.801	694.00	0.931	0.630
EM	QP	$-0.0002\times Val^2+0.2577\times Val-36.92$	0.743	612.04	0.821	0.812
	QBL	$54.946-0.0001\times(724.993-Val)^2$	0.704	717.74	0.962	0.9432
	LBL	$1.7177-0.0010\times(712.00-Val)$	0.834	712.00	0.954	0.0017
FCR	QP	$0.00001\times Val^2-0.0155\times Val+7.250$	0.854	736.25	0.987	0.0015
	QBL	$1.7254-0.000008\times(726.00-Val)^2$	0.829	718.74	0.964	0.0017
Average				694.81	0.931	

LBL: linear broken-line, QP: quadratic polynomial, QBL; quadratic broken-line, EW: egg weight, EM: egg mass, FI: feed intake, FCR: feed conversion ratio.

significantly affect egg shape index, egg density, and eggshell thickness.

Using the regression analysis for egg quality traits, two significant regression models were obtained (Haugh unit and albumen ratio). The best fit was achieved by the QP model (R^2 = 0.787, SSE = 2.598) for Haugh unit, and the estimated ratio was 0.932. The optimal dVal: dLys ratio for albumen percentage (0.938) was obtained by the QP model (R^2 = 0.664, SSE = 0.0003).

Table 4 – Egg quality criteria of laying hens fed with various dietary dVal: dLys ratios.

	Dietary SID Val: Lys							*p*-Values		
	0.82	0.86	0.90	0.94	0.98	1.02	SEM	ANOVA	L	Q
Egg shape index	0.774	0.778	0.782	0.770	0.787	0.775	0.016	0.72	0.90	0.33
Egg density	1.083	1.085	1.084	1.093	1.085	1.084	0.041	0.42	0.59	0.37
Albumen height (mm)	9.31^{c}	9.54^{abc}	9.49^{bc}	9.79^{abc}	9.96^{ab}	10.07^{a}	0.394	0.031	0.007	0.866
Haugh unit	95.26^{b}	95.76^{b}	98.76^{a}	98.97^{a}	97.23^{ab}	96.38^{b}	0.742	0.003	0.12	0.006
Yolk color index	7.37^{c}	7.57^{bc}	7.80^{ab}	8.03^{ab}	8.03^{ab}	8.20^{a}	0.358	0.0018	0.001	0.39
Albumen ratio	0.563^{b}	0.570^{b}	0.603^{a}	0.586^{a}	0.585^{ab}	0.579^{b}	0.008	0.019	0.171	0.013
Yolk ratio	0.271	0.258	0.266	0.268	0.252	0.261	0.013	0.921	0.548	0.903
Eggshell thickness (mm)	0.387	0.382	0.378	0.385	0.385	0.374	0.012	0.48	0.29	0.64
Yolk height (mm)	14.84^{b}	14.87^{b}	14.94^{b}	15.21^{b}	15.62^{a}	15.23^{ab}	0.373	0.045	0.008	0.69

a-c Means in a row with different superscripts are significantly different ($p<0.05$).

SEM: standard error of the means, L: linear, Q: quadratic.

The results of serum biochemical indicators are given in Table 5. Dietary SID Val levels had significant effects on serum albumin ($p<0.01$), uric acid ($p<0.01$), triglyceride ($p<0.05$), and a tendency on serum phosphorus ($p<0.10$). Serum albumin increased linearly up to the ratio of 0.98. Serum uric acid decreased quadraticaly up to the ratio of 0.94, and then increased at the ratios of 0.98 and 1.02. Serum triglyceride decreased linearly up to the ratio of 1.02. The dietary dVal: dLys levels had no significant effects on serum concentrations of total protein, glucose, cholesterol, phosphorus and calcium.

The serum biochemical indicators were also used to establish SID Val requirements. Among the variables tested, uric acid data fitted well to the aforementioned models. The SID Val requirements for minimum serum uric acid by the LBL, QP, and QBL models were 690.20, 702.50, and 725.57 mg/day, respectively. The best fit was attributed to the QP model (R^2 =0.927 and SSE = 0.009) for uric acid, and the respective ratio of SID Val: Lys was 0.942.

DISCUSSION

The findings of the current study showed that dietary Val supplementation (increasing dVal: DLys ratio) improved the performance (EW, EM, and FCR) of laying hens at the peak egg production phase. These results are consistent with recent studies (Harms & Russel, 2001; Peganova & Eder, 2002; Lelis *et al.*, 2014; Wen *et al.*, 2019; Jian *et al.*, 2021). However, Val supplementation had no significant effect on EP, which was in agreement with Azzam *et al*. (2015). Harmes & Russel (2001) indicated that rations with < 0.63% total valine can decrease the egg production rate, while the dVal level of the BD in the present study was higher (0.64% SID Val). Val is essential for egg production, and inadequate Val intake reduces egg production (Bregendahl *et al.*, 2008). Similarly, the insignificant effect of increasing dVal: dLys ratios on FI in the current experiment can be attributed to a higher level of dVal in the BD.

Table 5 – Effects of dietary SID Val: Lys ratios on serum biochemical indicators of laying hens (mg/dl).

	Dietary SID Val: Lys							*p*-Values		
	0.82	0.86	0.90	0.94	0.98	1.02	SEM	ANOVA	L	Q
Total protein (g/dl)	6.38	6.25	6.24	6.30	6.24	6.34	0.155	0.95	0.84	0.41
Albumin (g/dl)	1.42^{c}	1.40^{c}	1.42^{c}	1.48^{bc}	1.65^{a}	1.57^{bc}	0.041	0.001	0.001	0.40
Uric acid	4.97^{a}	4.69^{ab}	4.41^{bc}	4.26^{c}	4.40^{bc}	4.62^{b}	0.134	0.006	0.0051	0.0001
Glucose	260.80	261.70	251.200	262.00	253.10	263.10	8.62	0.62	0.94	0.36
Triglyceride	2424.83^{a}	2416.33^{a}	2293.30^{ab}	2244.11^{ab}	2217.75^{b}	2195.71^{b}	68.12	0.034	0.032	0.98
Cholesterol	162.86	177.00	169.25	185.60	181.57	184.33	12.72	0.58	0.14	0.67
Calcium	22.25	21.60	21.61	22.32	22.28	22.39	0.36	0.13	0.17	0.18
Phosphorus	6.88	6.74	7.46	7.52	8.23	6.73	0.52	0.073	0.28	0.07

$^{a-c}$ Means in a row with different superscripts are significantly different ($p<0.05$). SEM: standard error of the means, L: linear, Q: quadratic.

The SID Val requirements were determined to be 694.00, 694.00, and 736.25 mg/day for EW, EM, and FCR, respectively. These values were corresponding to dVal: dLys ratios of 0.931, 0.931, and 0.987 for EW, EM, and FCR; respectively. The SID Val requirement determined in this study (mean = 694.81 mg/day, or 0.727 %) was higher than that of Wen *et al*. (2019), Lelis *et al*. (2014), Bregendahl *et al*. (2008), Peganova & Eder (2002), Harms & Russel (2001), and NRC (1994). The estimated requirement was close to the Hy-Line W36 recommendation (704 mg/ day). The inconsistency of Val requirement in different studies may be explained by hen age (production phase), genetic potential of modern layers, hen strain, type of the BD, levels of other BCAAs in the BD, the method of expressing requirement (total vs. digestible), and the statistical methods (Macelline *et al*., 2021). The main reason for higher Val requirement in the current experiment can be attributed to hen's age (peaking phase) and high EM output in comparison to the previously mentioned studies. The obtained average hen day egg production (92%) was close to the 94 % indicated by the Hy-Line W36 management guide. Literature review revealed that few experiments were conducted in the peaking phase of laying hens. It is essential to supply adequate levels and a proper ratio of dVal: dLys and other indispensable amino acids in the peaking diet of laying hens to reach the maximum production potential (Lelis *et al*., 2014).

The dVal: dLys ratio obtained in the present experiment (0.931) was identical to that of Bregendahl *et al*. (2008, 0.93), and close to the ratios reported by Lelis *et al*. (2014, 0.92), Brazilian tables (2017, 0.9), and Hy-Line W36 (2020, 0.88). It seems that recent experiments reporting consistent ratios for dVal: dLys, and the mean ratio of recent experiments (0.91) can be used in ration formulation in different production phases.

Due to economic concerns, determining the amino acid requirements of laying hens as mg of amino acids per gram of EM has been attracting the attention of researchers. In this study, the optimum amount of dVal per gram of egg mass was calculated to be 12.79. This finding is consistent with the value of 12.2 reported by Wen *et al*. (2019). However, Harms & Russell (2001) reported a Val requirement of 13.1 per gram of EM. Decreasing Val need per gram of EM is indicative of the improved utilization of amino acids in modern laying hens.

Our results showed that albumen height, Haugh unit, yolk color, albumen ratio, and yolk height increased linearly or quadratically by increasing the dietary dVal: dLys ratio. These results are in contrast with previous reports. Wen *et al*. (2019) found a decreasing pattern in Haugh unit as Val concentration increased. Lelis *et al*. (2014) and Peganova & Eder (2002) reported that Haugh unit and albumen height were not affected by dietary Val concentrations. One of the main reasons for this discrepancy is the age of hens in different experiments. The age of hens at the start of the above mentioned experiments were 40 weeks or more (after peaking phase), while it was 28 weeks in the present experiment. Hen age is the most important factor affecting egg quality. Albumen quality decreases rapidly with advancing hen age (Williams, 1992). The above mentioned experiments were conducted after peaking phase, in which Haugh unit and albumen ratio decrease with advancing hen age. Moreover, the highest contents of amino acids in egg proteins were related to leucine, Lys, and Val, which constituted 8.3%, 7.1%, and 6.5% of egg proteins, respectively. An interesting point is that Val is one of the most abundant amino acid in ovomucin, and there is a positive correlation between egg ovomucin content and Haugh unit (Romanoff & Romanoff, 1949). Ovomucin is a fibrous protein that plays an important role in the quality of the egg white, maintains it in the form of a firm gel, and gives it shape and strength. Increasing the dietary dVal: dLys ratio linearly increased the yolk color index. Jian *et al*. (2021) reported that Val supplementation significantly increased duodenal digestive enzymes (trypsin and lipase) activity in a local breed of laying hens. Increased digestive enzyme activity may be helpful in digestion and absorption of dietary carotenoid pigments. Yolk height increased in response to the dietary Val supplementation. Higher yolk height is indicative of a strong yolk vitelline membrane. Weak vitelline membranes impose economic losses in the egg processing factories, since the yolk and white separation becomes difficult after the rupture of the yolk membrane (Visscher, 2019).

The results of this study indicated that an increased dVal: dLys ratio results in a linear increase in serum albumin, which is in line with the results obtained by Azzam *et al*. (2015). Albumin is an important long-term indicator of the blood protein status (Laborde *et al*., 1995). Smith (1978) suggested that serum albumin is the main storage protein in the blood of laying hens. Serum albumin degrades during the synthesis of egg white's proteins in the oviduct, where high levels of amino acids are required, which results in serum albumin reduction. The increase in serum albumin with the addition of Val in this experiment can be due to the prevention of the breakdown of albumin to supply the deficiency of Val. Dibner & Ivey (1990) reported that a response of the liver during stress, such as deficiency of an amino acid, is to reduce the synthesis of albumin. Therefore, decreasing valine in the ration decreases blood albumin, and the decreased levels of blood proteins and albumin negatively affect the formation of eggs in laying hens (Azzam *et al.*, 2015). Serum uric acid decreased quadraticaly by increasing the dVal: dLys ratio. Serum levels of uric acid are affected by the balance of dietary amino acids. Chi & Speers (1976) reported that increased levels of dietary synthetic amino acids may decrease uric acid, attributing this to a better dietary protein balance. Fernandez *et al*. (1996) stated that utilizing synthetic amino acids and providing more balanced rations leads to lesser release of nitrogen into the environment. Chi & Speers (1976) reported that the increased lysine content of rations can reduce serum levels of uric acid, which is similar to our findings. In the study of Azzam *et al*. (2015), dietary valine contents did not affect the serum uric acid of layers at the post peak phase. Another study by Azzam *et al*. (2011) indicated no changes in blood uric acid levels when threonine was added to the experimental rations. Increased ratios of dVal: dLys had a significant impact on serum triglyceride of laying hens, with the level of serum triglycerides reducing linearly with the increase of the Val content of rations. A large proportion of serum triglycerides in laying hens are present as yolk lipoproteins. These decreases in serum triglycerides in laying hens can be related to the increased uptake of yolk precursors by ovarian follicles. No research has been conducted on the effect of dietary Val on serum triglyceride levels, and further research is warranted.

CONCLUSIONS

In conclusion, Val supplementation improved EW, EM, FCR, and egg quality traits (Haugh unit, yolk color, yolk height, and albumen ratio) of laying hens at the peaking phase. The daily SID Val requirement of 695 mg (dVal: dLys ratio of 0.93) is recommended for Hy-Line W-36 laying hens at the peak egg production phase. The optimum ratio of dietary digestible valine: lysine for the other commercial strains should be further explored.

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Author contributions

Conceptualization, MHSH and MN; methodology, MHSH and HMK; software, MHSH and HMK; validation, MN, MHSH and HMK; formal analysis, HMK and MHSH; investigation, MN; resources, MH; data curation, MN; writing—original draft preparation, MN; writing—review and editing, MHSH and HMK and MH; visualization, MN and HMK; supervision, MHSH; project administration, MH; funding acquisition, MHSH and MH. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data will be available upon request from the corresponding author.

Conflicts of interest

The authors declare that they have no conflict of interest associated with this publication.

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