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Digestible Arginine Requirements in Hy-Line W-36 Laying Hens: Effects on Performance, Egg Characteristics, and Plasma Parameters During 40 to 46 Weeks of Age

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Abstract
This study was conducted to estimate digestible arginine (Arg) requirements for performance and egg quality parameters in Hy-Line W-36 laying hens from 40 to 46 weeks of age. A total of 150 laying hens were arranged in a completely randomized design with 5 treatments, 5 replicates and 6 hens in each. These treatments included 0.81, 0.86, 0.91, 0.96, and 1.01 % digestible Arg. At the end of experiment, data were obtained in performance, egg quality, and quantity parameters as well as plasma levels of cholesterol, uric acid, globulin, and insulin-like growth factor (IGF-1). Results have shown that feed conversion ratio, egg production, and egg mass improved by supplementing 0.86 digestible Arg in the diet compared to other treatments ($P < 0.05$). Moreover, these items significantly affected by the interaction of treatments and weeks. In the last three weeks of this experiment, the use of 0.86, 0.91, 0.96, and 1.01 % digestible Arg significantly increased egg mass and egg roduction. Plasma concentrations of globulin, cholesterol, and uric acid were not affected by dietary Arg levels. However, a significant increase in plasma IGF-1 was shown by supplementation of % 0.91 Arg ($P < 0.05$). Based on quadratic equations, the optimum levels of digestible Arg for egg production, egg mass, feed conversion ratio, and IGF-1 were 0.917, 0.917, 0.908, and 0.970%, respectively.

Introduction

The arginine amino acid (Arg) is an important substrate for protein metabolism and is also an essential amino acid in birds' nutrition. Therefore, the birds have an absolute need for L-Arg and are highly dependent on its dietary supplementation (Miao *et al*, 2017). The Arg also serves as a substrate for the generation of creatine, polyamines, L-proline, various hormones, and nitric oxide (NO), which serves multiple roles within the immune system (Khajali and Wideman, 2010). It was reported that reduced oviduct weight and the number of small yellow follicles, also increased yolk color and immunoglobulin Y (IgY) content of the eggs by arginine supplementation of diet (17 mg L-Arg/kg diet) in compression to other treatment. In contrast, no effects were observed on laying performance, egg quality, and blood parameters (Yang *et al.*, 2016). Liu *et al* (2014) have shown that an improvement in the innate and acquired immune system of chickens fed with Arg-supplemented diets and also challenged with Salmonella .

Several studies have shown that the positive impact of dietary arginine supplementation higher than the recommended levels on the performance of laying hens (NRC, 1994). Youssef *et al.* (2016) stated that Arg supplementation at a higher level than NRC could significantly increase egg production and egg mass during the whole experimental period. There was at least 6% of extra egg production over control. Fernandes *et al.* (2009) have suggested that dietary supplementation with Arg at higher levels than recommended for the starter phase may be necessary for improved muscle development in broilers. Also, Silva *et al* (2012), reported an increase in egg production and egg mass by L-Arg dietary supplementation. Dietary Arg requirements of broilers may not be adequate and enough to support

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maximal growth, immune function, avoid pulmonary hypertension at high altitudes, cold environmental conditions, disease challenges, and management conditions (Khajali and Wideman, 2010). Although that L-Arg can stimulate protein synthesis, this is demonstrated in some in vitro studies in chickens or pigs (Yuan *et al*., 2015). The L- Arg improved weight gain and feed intake, especially in growing chickens and pullets independent of genetic background (Lieboldt *et al*., 2015). Amino acids are major regulators of growth and protein metabolism and diets deficient in a specific limiting amino acid could lead to decreased protein accretion (Tesseraud *et al*., 1999). Therefore, this study managed to determine the requirement of Arg digestible in laying hens by regression equations and evaluate the effects of Arg on performance, blood parameters, and IGF-1 status.

Materials and Methods

Birds and Housing

In this experiment, 150 laying hens of Hy-Line W-36 strains were allocated to 5 treatments, 5 replicates, and 6 hens in each. The experiment duration was carried out from 40 to 46 weeks of age.. The temperature was kept at around 18 to 22°C in the poultry house. The room humidity varied from 52 to 64%, and the lighting program was set to 16 hours of light and 8 hours of darkness during the experiment period.

Experimental diets

Two weeks before the experiment (38 to 39 weeks), was as an adaptation period. The experimental diets (Table 1) were adjusted according to the nutritional recommendation guided by Hy-Line W-36 commercial layers (2015). Diets in mash form were offered *ad libitum* and birds had free access to water throughout the experiment. Experimental treatments were obtained by adding 0.81, 0.86, 0.91, 0.96, and 1.01% of digestible Arg (98% of purity, Merck, Germany) . Dry matter, ash, crude protein, crude fiber, and crude fat content of raw materials in dietary treatments (corn, soybean meal, barley, and corn gluten) were measured according to the AOAC (2000). Feed intake was provided twice per day (morning and evening) the residual of replicate feed was recorded at the end of each week and replaced by a new diet. There was no mortality, therefore, weekly feed intake was recorded based on the day hen by the following formula (Harms and Racell, 2001):

Day hen = 7 × number of alive hens

Means of feed intake for each hen per day = Weekly alive hens feed intake/day hens

Egg mass = egg weight × production percentage

FCR = Feed intake/Egg mass

Also, the amino acid profile of the feed ingredients was determined by Near-Infrared Reflectance Spectroscopy (NIRS) and standardized ileum digestible amino acids estimated by coefficients of Degosa Evonik (Amino-Dat. 5).

Table 1. Basic dietary components along with chemical analysis (as %)

Corn	46.56
Soybean meal	19.29
Corn gluten meal	4.60
Barley	13.00
Soybean oil	3.22
Dicalcium phosphate	2.34
Oyster shell Powder	10.00
Salt	0.40
Mineral supplement	0.25
Vitamin supplement	0.25
D, L-Methionine	0.09
Chemical analysis	
Metabolism energy (kcal/kg)	2822
Crude protein (%)	16.37
Calcium (%)	4.40
Available P (%)	0.55
Sodium (%)	0.18
Chlorine (%)	0.29
Lysine (%)	0.71
Methionine	0.36
Methionine + Cystine (%)	0.60
Threonine (%)	0.50
Tryptophan (%)	0.15
Arginine (%)	0.81
Valine (%)	0.69
Isoleucine (%)	0.59

Provided per kg of the mineral supplement: 70 mg of manganese (oxide), 60 mg of zinc (oxide), 60 mg of iron (sulfate), 8 mg of copper (sulfate), 1.1 mg of iodine (calcium iodate), 0.15 milligrams of cobalt and 0.25 milligrams of selenium.

Each kilo of vitamin supplement: 10,000 units of vitamin A, 2500 international units of vitamin D_3, 20 international units of vitamin E, 3 milligrams of vitamin K_3, 2 mg thiamine, 5 mg of riboflavin, 12 mg of pantothenic acid, 40 mg niacin 5200 mg choline chloride, 5 mg Pyridoxine, 0.015 mg of cobalamin, 0.05 grams of biotin, 400 mg choline chloride, 0.75 mg of folic acid.

Eggs quality characteristics

To evaluate the egg characteristics, two eggs were selected randomly from each replicate on two final days of each week and transferred to the laboratory. Initially, the length and width of each egg were measured to calculate the shape index, i.e., (width/length) × 100. To examine the characteristics of the eggshell, the shells were washed, then placed in an oven at 65°C for 24 hours, and weighed. The thickness of the shells was measured by a digital micrometer (INSIZE, Taiwan) with a precision of 0.001 mm (Harms and Russell, 2001), egg weight (EW), and albumen height (AH) were measured to calculate the Haugh Unit as the following formula.

$HU = 100 \text{ Log } (AH + 7.75 - 1.7 \, EW^{0.37})$.

The yolk color was recorded by the color spectrum of the Rosh unit. Further, characteristics such as yolk index (YI) and shell ratio (SR) were calculated from the raw data by the following formulas (Altan *et al*., 1998; Keener *et al*., 2006).

SR = shell weight/Egg weight ×100

YI = yolk height/yolk diameter ×100

Plasma Chemical Analysis

To evaluate the effects of amino acid levels of Arg on plasma biochemical parameters, at the end of the experiment (46 weeks), two hens from each replicate were randomly selected and blood samples were taken from their vein's wings. Heparin anticoagulant tubes were used to prevent coagulation of blood samples. Immediately after authorization, the plasma samples were centrifuged at 4472×*g* for 20 minutes and stored at -20°C until analysis. In this study, four parameters of plasma, including cholesterol, uric acid, globulin, and IGF-1, were recorded according to common colorimetric methods (Spectrophotometer).

Statistical analysis

This experiment was conducted in a completely randomized design (CRD) with 5 treatments, 5 replicates, and 6 hens in each. Production characteristics including egg weight, egg production, feed intake, egg mass, feed conversion ratio, body weight change and egg quality, and plasma parameters, containing cholesterol, uric acid, globulin, and IGF-1 concentrations were analyzed as repeated measures. Means of treatments with significant effects in the analysis of variance were compared based on Duncan at a significance level of 0.05. Orthogonal polynomial tests were also performed to evaluate the response curves. Digestible Arg requirements for the traits, with significant treatment effects and significant quadratic polynomial responses, were estimated by derivation of quadratic regression models. The data were analyzed by SAS (2008).

Results

Performance

The effect of experimental treatments on the performance of laying hens during the whole production period is presented in Table 2. No significant differences were found between treatments on feed intake, egg weight, and body weight change in the whole period ($P > 0.05$). In the analysis of feed intake, the linear, quadratic and cubic regressions were not significant ($P > 0.05$). There were no significant differences in body weight between different treatments in the whole period of production ($P > 0.05$). However, no response was shown in egg weight by dietary treatments. No significant differences were observed by analysis of the linear, quadratic and cubic regression during all weeks in egg weight ($P > 0.05$).

Table 2. Effect of dietary arginine levels on performance traits during 40 to 46 weeks of age

Dietary Arg level (%)	Feed intake (g/day)	Egg mass (g/day)	Egg production (%)	FCR [1] (g/g)	Egg weight (g)	CBW [2] (g/period)
0.81	93.90	47.37[c]	77.28[c]	1.941[a]	61.15	0.15
0.86	93.09	54.34[a]	89.18[a]	1.712[b]	60.96	0.16
0.91	95.27	50.60[b]	82.72[b]	1.863[a]	61.08	0.13
0.96	94.66	50.06[b]	81.77[b]	1.880[a]	61.15	0.14
1.01	94.99	50.58[b]	82.65[b]	1.864[a]	61.10	0.15
SEM [3]	1.512	0.452	0.81	0.028	0.608	0.040
P-value [4]						
Treatment	0.845	<0.0001	<0.0001	0.0003	0.999	0.244
L. R	0.441	0.153	0.246	0.858	0.959	0.858
Q. R	0.938	<0.0001	<0.0001	0.018	0.919	0.018
C. R	0.674	<0.0001	<0.0001	0.0001	0.826	0.0001
Week	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment×Week	0.0022	<0.0001	<0.0001	0.0027	0.9088	0.0027

[1] FCR, Feed conversion ratio.

[2] CBW, Changes in body weight.

[3] *SEM*, Standard error of means.

[4] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

The highest and the lowest value of egg production and egg mass were found in 0.86% and 0.81% Arg treatments, respectively ($P < 0.05$). The use of 0.86% Arg significantly improved the feed conversion ratio. However, these differences were not considered due to the significant interaction of treatment × week during the whole experimental period. Therefore, it should be statistical analysis separately in different weeks (Tables 3, 4, and 5). In addition, significant quadratic and cubic regression equations were found in egg production (Table 6), egg mass, and feed conversion ratio ($P < 0.05$). The effect of treatment on egg production, egg mass, and feed conversion ratio were significant in the fourth, fifth, sixth, and seventh weeks. In the 4th week, egg production significantly decreased in a level of 0.81% Arg compared with other treatments.

Table 3. Effects of dietary arginine levels on egg production (%) of laying hens in different weeks (40 to 46 weeks)

Dietary Arg level (%)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
0.81	93.34	87.14	83.33	76.66^{b}	71.43^{c}	64.28^{c}	64.76^{c}
0.86	91.91	89.52	82.85	86.66^{a}	90.95^{a}	90.47^{a}	91.90^{a}
0.91	91.91	87.62	84.76	85.71^{a}	80.95^{b}	74.76^{b}	73.33^{b}
0.96	86.19	90.47	82.85	84.76^{a}	78.09^{b}	75.23^{b}	74.76^{b}
1.01	90.00	91.43	83.33	84.28^{a}	80.47^{b}	74.28^{b}	74.76^{b}
SEM [1]	0.763	2.386	1.826	1.587	1.850	1.564	2.064
P-value [2]							
Treatment	0.0781	0.6741	0.9440	0.0018	<0.0001	<0.0001	<0.0001
L. R	0.0388	0.2215	0.9997	0.0152	0.3821	0.3476	0.6670
Q. R	0.4781	0.8333	0.7827	0.0021	0.0008	<0.0001	<0.0003
C. R	0.1621	0.7556	0.9992	0.0338	<0.0001	<0.0001	<0.0001

[a-c] Different letters indicate significant differences between groups in Duncan's test ($P < 0.05$).
[1] *SEM*, Standard error of means.
[2] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

Table 4. Effects of dietary arginine levels on egg mass (g/day) of laying hens in different weeks (40 to 46 weeks)

Dietary Arg level (%)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
0.81	58.77	54.95	51.35	46.07^{b}	42.61^{c}	38.19^{c}	39.63^{c}
0.86	58.40	55.84	50.26	52.41^{a}	54.66^{a}	53.03^{a}	55.78^{a}
0.91	58.50	54.42	52.31	51.96^{a}	48.69^{b}	44.27^{b}	44.10^{b}
0.96	54.64	56.90	50.85	50.87^{a}	47.06^{b}	44.91^{b}	45.14^{b}
1.01	57.09	57.80	50.68	51.05^{a}	48.54^{b}	43.95^{b}	44.89^{b}
SEM [1]	1.186	1.437	1.153	0.988	1.170	1.019	1.401
P-value [2]							
Treatment	0.1211	0.4679	0.7615	0.0014	<0.0001	<0.0001	<0.0001
L. R	0.0728	0.1527	0.8422	0.0138	0.2625	0.3042	0.9804
Q. R	0.7097	0.4757	0.7013	0.0022	0.0010	<0.0001	<0.0001
C. R	0.1353	0.8750	0.6162	0.0177	<0.0001	<0.0001	<0.0001

[a-c] Different letters indicate significant differences between groups in Duncan's test ($P < 0.05$).
[1] *SEM*, Standard error of means.
[2] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

Table 5. Effects of dietary arginine levels on feed conversion ratio (g/g) of laying hens in different weeks (40 to 46 weeks)

Dietary Arg level (%)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
0.81	1.700	1.815	1.965	2.011^{a}	1.955^{a}	2.123^{a}	2.011^{a}
0.86	1.651	1.722	1.852	1.790^{b}	1.733^{b}	1.688^{c}	1.590^{b}
0.91	1.703	1.833	1.824	1.864^{ab}	1.901^{a}	1.963^{b}	1.932^{a}
0.96	1.804	1.733	1.883	1.913^{ab}	1.950^{a}	1.970^{b}	1.946 ^{a}
1.01	1.721	1.705	1.924	1. 872^{ab}	1.892^{a}	1.971^{b}	1.934^{a}
SEM [1]	0.051	0.053	0.073	0.046	0.041	0.048	0.037
P-value [2]							
Treatment	0.3120	0.3901	0.6814	0.0449	0.0076	<0.0001	<0.0001
L. R	0.2153	0.2295	0.9588	0.3473	0.5236	0.9131	0.0990
Q. R	0.9141	0.6912	0.2037	0.0632	0.2170	0.0029	0.0023
C. R	0.0963	0.4622	0.4990	0.0178	<0.0011	<0.0001	<0.0001

[a-c] Different letters indicate significant differences between groups in Duncan's test ($P < 0.05$).
[1] *SEM*, Standard error of means.
[2] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

In 5, 6, and 7 weeks, egg production significantly decreased with 0.81% Arg, but reverse trend was noted in 0.86% Arg treatment. The egg mass significantly decreased with the level of 0.81% Arg in week 4, but there were no significant differences between the other treatments. In 5, 6, and 7 weeks, egg mass significantly decreased with the level of 0.81% Arg and increased significantly with 0.86% Arg. In the 4th and 6th weeks, the feed conversion ratio significantly increased by 0.81 and decreased by 0.86% Arg. In 5 and 7 weeks, FCR decreased significantly with a level of 0.86% Arg, but no significant differences were shown by other treatments.

Table 6. Effect of dietary arginine levels on the egg quality of laying hens (from 40 to 46 weeks of age)

Dietary Arg level (%)	Shape index (%)	Yolk index (%)	Yolk color (Roche)	Haugh unit	Shell thickness (mm)	Shell ratio (%)
0.81	75.67	44.80	6.82	83.83	0.470	8.84
0.86	75.28	45.59	6.82	82.89	0.481	8.73
0.91	75.08	45.37	7.10	83.64	0.502	9.05
0.96	75.94	45.47	6.75	84.03	0.473	9.00
1.01	73.91	45.89	6.78	83.57	0.484	9.33
SEM [2]	0.883	0.660	0.125	0.689	0.007	0.177
P-value [2]						
Treatment	0.5481	0.8268	0.3155	0.8136	0.1754	0.2079
L. R	0.3178	0.3317	0.6627	0.7873	0.9563	0.0400
Q. R	0.5057	0.8701	0.2332	0.8258	0.1162	0.4541
C. R	0.2839	0.5315	0.8029	0.2567	0.3306	0.9440
Week	0.6030	<0.0001	<0.0001	<0.0001	<0.0001	0.0281
Treatment×Week	0.3762	0.2862	<0.0001	0.1708	0.0036	0.0024

[a-c] Different letters indicate significant differences between groups in Duncan's test ($P < 0.05$).
[1] *SEM*, Standard error of means.
[2] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

Egg characteristics

The high level of dietary Arg (% 0.96) leads to an increased yolk color (Table 7). The analysis of linear, quadratic, and cubic regression relationships for all egg characteristics was not significant in the experimental whole period ($P > 0.05$).

Blood parameters

The effect of treatments on, globulin, IGF-1, uric acid, and cholesterol of birds are represented in Table 8. No significant effect of Arg treatments was found on plasma globulin, uric acid, cholesterol, and IGF-1 by linear, quadratic, and cubic regression. In addition, a significant increase was found in plasma IGF1 by 0.91% Arg in control significant decrease was shown by 0.81 Arg in this respect, in comparison to other treatments ($P < 0.05$).

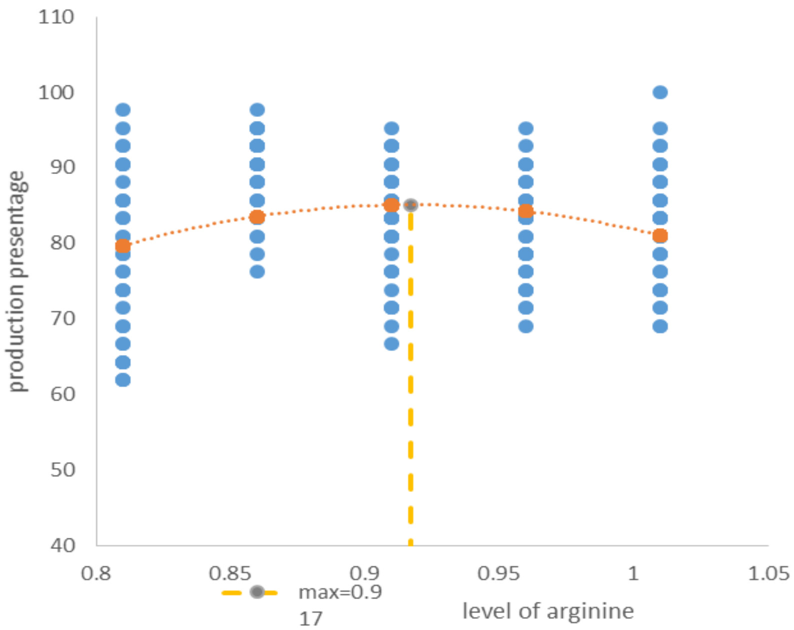

Figure 1. Dietary requirement of arginine (%) to maximize egg production.

Table 7. Effects of dietary arginine levels on Yolk color, Shell thickness, and Shell ratio in different weeks (40 to 46 weeks).

	Yolk color (Roche)				Shell thickness (mm)				Shell ratio (%)			
Dietary Arg level (%)	Week40	Week42	Week44	Week46	Week40	Week42	Week44	Week46	Week40	Week42	Week44	Week46
0.81	6.30	6.70^{bc}	7.50	6.80^{abc}	0.480	0.477^{a}	0.50^{0ab}	0.463^{b}	8.58	8.97^{a}	8.95	8.87^{ab}
0.86	6.00	7.20^{ab}	7.00	7.10^{ab}	0.491	0.496^{a}	0.541^{a}	0.542^{a}	9.03	8.25^{b}	9.23	8.42^{b}
0.91	6.10	7.60^{ab}	7.20	7.50^{a}	0.495	0.497^{a}	0.532^{a}	0.534^{a}	8.87	9.12^{a}	8.92	9.29^{ab}
0.96	5.70	7.70^{a}	7.10	6.50^{bc}	0.488	0.465^{b}	0.504^{ab}	0.501^{ab}	9.02	8.84^{ab}	8.24	9.88^{a}
1.01	5.60	5.60^{c}	7.50	6.20^{c}	0.519	0.481^{a}	0.483^{b}	0.484^{b}	8.78	9.31^{a}	9.14	10.07^{a}
SEM [1]	0.125	0.154	0.223	0.250	0.009	0.012	0.015	0.015	0.162	0.200	0.320	0.403
P-value [2]												
Treatment	0.2121	0.0003	0.4020	0.0141	0.1080	0.0329	0.0472	0.0472	0.2870	0.0151	0.2487	0.0476
L. R	0.0197	0.0001>	0.8890	0.0346	0.1517	0.2436	0.0570	0.9634	0.4506	0.0604	0.5652	0.0065
Q. R	0.9010	0.0723	0.0881	0.0119	0.1970	0.2722	0.0260	0.0203	0.0892	0.1145	0.4771	0.5097
C. R	0.8830	0.8403	0.7802	0.4585	0.0463	0.1039	0.2314	0.5259	0.6764	0.1981	0.0442	0.1959

[a-c] Different letters indicate significant differences between groups in Duncan's test ($P < 0.05$).
[1] *SEM*, Standard error of means.
[2] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

Table 8. Effect of arginine amino acid level on some plasma parameters

Dietary Arg level (%)	IGF-1 [1] (µg/dL)	Globulin (ng/ mL)	Uric acid (mg/dL)	Cholesterol (mg/dL)
0.81	30.75[c]	2.34	8.05	100.70
0.86	36.86[bc]	2.22	5.02	135.70
0.91	55.18[a]	2.36	5.00	131.00
0.96	38.85[bc]	3.02	4.13	175.60
1.01	51.08[ab]	2.34	4.45	123.30
SEM [2]	5.416	0.348	0.949	26.062
P-value [3]				
Treatment	0.0322	0.5125	0.0576	0.3880
L. R	0.0173	0.4757	0.0139	0.3141
Q. R	0.4275	0.6499	0.1073	0.2135
C. R	0.4550	0.1615	0.5512	0.4956

[a-c] Different letters indicate significant differences between groups in Duncan's test ($P < 0.05$).
[1] IGF-1, Insulin-like growth factor.
[2] *SEM*, Standard error of means.
[3] L. R, linear regression; Q. R, Quadratic regression; C. R, Cubic regression.

Table 9. The quadratic regression equation and level of required arginine

Traits	Quadratic regression equation	Estimated Arginine Requirement (dig %)[1]
Egg Production	Y= -312.10 + 866.28X - 472.32X2	0.917
Egg mass	Y= -182.23 + 510.49X - 278.15X2	0.917
FCR [2]	Y= 8.33 - 14.35X + 7.90X2	0.908
IGF-1[3]	Y= 75.98 + 33.073X - 14.04X2	0.970

[1]Requirements base Digestible
2 Feed conversion ratio.
[3] Insulin-like growth factor 1

Digestible Arg requirement

Estimation of digestible Arg requirement was carried out by using the quadratic regression method. The equivalence of the quadratic regression and the required levels of Arg are shown in Table 9. The estimated levels of Arg requirements for egg production, egg mass, feed conversion ratio and IGF-1 were 0.917, 0.917, 0.908, and 0.970%, respectively (Figures 1, 2, 3, and 4 on the curves, respectively).

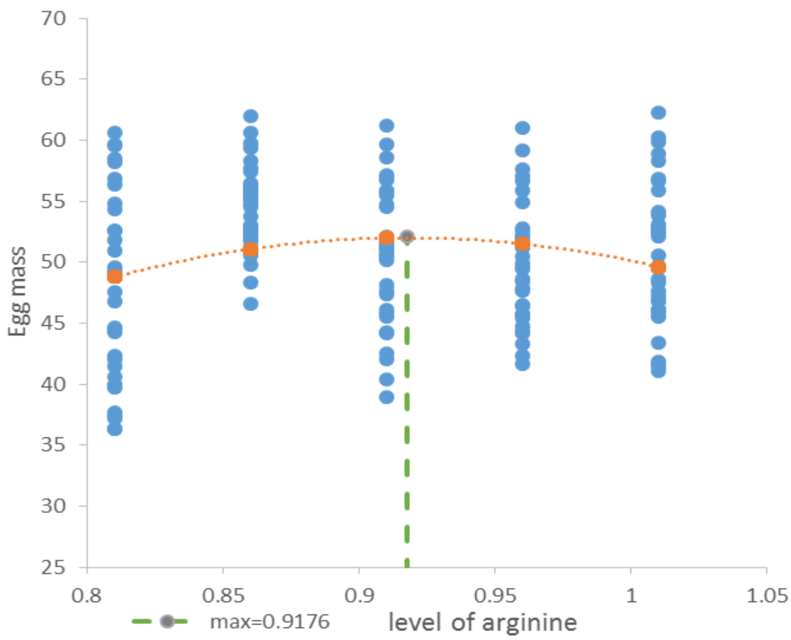

Figure 2. Dietary requirement of arginine (%) to maximize egg mass.

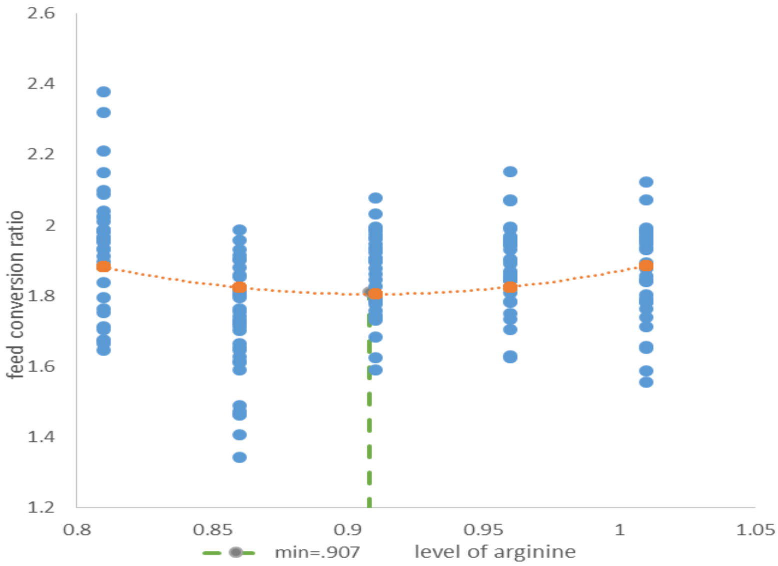

Figure 3. Dietary requirement of arginine (%) to minimize feed conversion ratio.

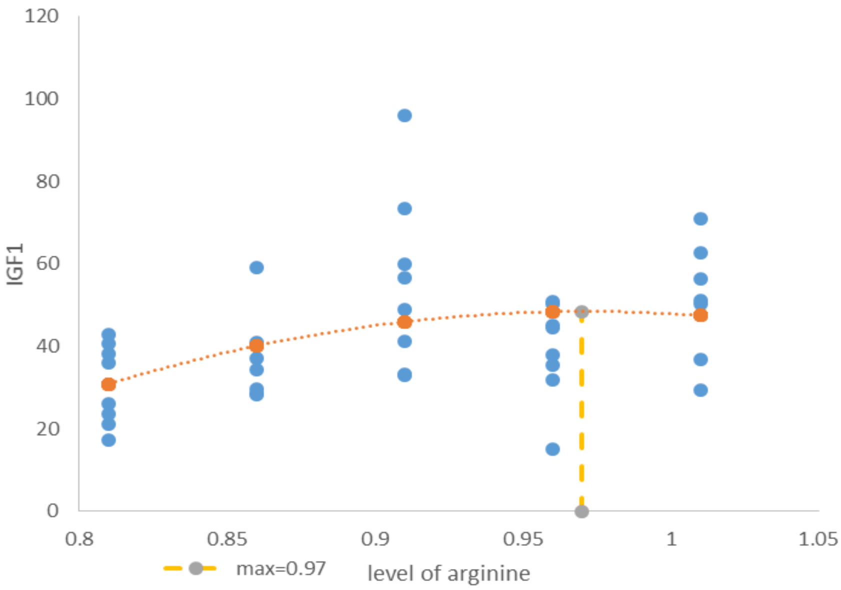

Figure 4. Dietary requirement of arginine (%) to maximize blood plasma IGF-1 concentration.

Discussion

Poultry performance is affected by the essential amino acids. However, high levels of any of the amino acids may have a negative effect on performance and the antagonism between amino acids such as Arg and lysine could be observed (D'mello, 2003). Analysis of data on feed conversion ratio did not show any significant differences between experimental treatments during the whole experimental period. In contrast in the final weeks, significantly different levels of Arg influenced the feed conversion ratio, and these results were confirmed by Lieboldt *et al.*, (2016) and Jahanian and Khalifeh-Gholi, (2018) who have shown negative effects of dietary Arg reduction on feed conversion ratio.

Analysis of data on the percentage of production in the whole experimental period did not show any differences among treatments. But there were significant differences in the increase of production in the fourth, fifth, sixth, and seventh weeks by % 0.86 of Arg. Increasing production in some research may be due to the improvement of the amino acid balance in ration and Arg intake of chickens for maximum production.

On the other hand, an increase in the level of Arg may increase the secretion of the LH hormone, followed by an increase in egg production (Basiouni, 2009). In contrast higher levels of Arg resulted in a reduction in the percentage of production. Increased egg production by increasing Arg levels could be obtained but a higher Arg level more than the requirement status could lead to decrease egg production this may be related to Arg and lysine antagonism. Also, the Arg amino acid of the N-molecule is highly integrated with the structure, hence the poultry require a lot of energy for the metabolism process and the removal of excess Arg. Further increases in the level of Arg did not increase the amount of egg production and were consistent with the results of Najib and Basiouni (2004) and Basiouni *et al*., (2006). It has been reported that excess Arg in the diet increases uric acid excretion. On the other hand, a molecule of glycine is excreted with the removal of any uric acid molecule. The glycine amino acid is synthesized in the poultry body to a limited extent, and when poultry encounters an excess of uric acid, it does not meet the needs of tissues and organs (Corzo *et al*., 2004). Following this, may decrease the percentage of egg production.

Analysis of data on average egg weight in different weeks and the whole experiment period did not show any significant differences among experimental treatments. The results of the mean egg weight in response to the Arg supplement in this study were compared with previous studies by Carvalho *et al*. (2012), Yuan *et al*. (2015), and Youssef *et al*. (2016). In an analysis of data on the average egg mass over the whole trial period, there were no significant differences between experimental treatments. According to previous research results, higher levels of Arg reduce the average egg mass. Achieving these results was not unexpected. Because the egg mass depends on the percentage of production and the weight of the egg production. An analysis of feed intake data did not show any significant differences among experimental treatments during the whole period of study. The results of this study on feed intake are consistent with the results by Rubin *et al*. (2007), Carvalho *et al*. (2012), and Youssef *et al*. (2016).

There is limited literature considering the effects of Arg acid on egg quality. Analysis of data on quality traits did not show a significant difference in the characteristics of shape index, yolk index, yolk color, eggshell, Haugh unit, and shell weight. According to Carvalho *et al*. (2012) and Yang *et al*. (2016) results, increasing the level of Arg in laying hens did not have any significant effect on the egg quality

However, Carvalho *et al*, (2015) have reported that increasing levels of digestible lysine and Arg lead to reduced eggshell quality and albumen solids, respectively. Yang *et al*. (2016) also indicated that increased levels of dietary Arg increased the yolk color. These results are consistent with the results of the present study. Yuan *et al*. (2015) observed no significant differences in egg characteristics by increasing the Arg level in the diet, but an exception was shown by significantly improved Haugh unit. De Lima and da Silva (2007), Silva *et al*. (2012), and Youssef *et al*. (2016) no significant differences were recognized in the internal egg quality characteristics by increasing Arg levels. Results of the present study have shown that increasing the levels of Arg leads to an increase in the shell thickness of the eggs, by improving mineral deposition in the bone and influencing the metabolism of the minerals Carlisle (1986); Visser and Hoekman (1994).

In the present study, no significant differences were indicated in plasma cholesterol by increasing dietary Arg levels in laying hens, However, the study of Emadi *et al*. (2010) and Fouad *et al*. (2013) were inconsistent with our results. They have stated that dietary Arg leads to decrease plasma triglyceride and total cholesterol concentrations in broilers fed Arg-supplemented diets. Fouad *et al*. (2013) have shown that the Arg mechanism effect on plasma cholesterol is still not well known and requires further studies. There are very few studies that have considered the effects of Arg on IGF-1 plasma levels. Arg is considered a secretagogue, increasing the level of IGF-A in the blood (Silva *et al*., 2012). In this experiment, analyzing data on plasma IGF-1 levels showed that increasing the level of Arg in the diet increases the level of this parameter in the blood plasma. These results agree with the results of the study by Yu *et al*., (2018). They have indicated that increased levels of Arg in the plasma increase the secretion of the hormone IGF-1 and increase its level in the blood plasma. It has been reported that increasing the amount of Arg in the feed increases the synthesis of ornithine. Turnitin increases the secretion of the growth hormone by stimulating the pituitary gland (Isidori *et al*. 1981; Sugino *et al*. 2008). Analysis of data on plasma globulin levels has shown that increased levels of dietary Arg had no significant effect on plasma globulin levels.

The dietary requirement of L-Arg, noted by the NRC is not sufficient to maximize the performance of white and brown-egg-laying strains (Yuan *et al*., 2015). Schutte *et al*., (1994) have stated that the estimation of nutrient requirements may depend on the biological response and quantity of the requirement. In this study, the quadratic equations showed that digestible Arg requirement for maximum egg mass, production percentage, and insulin-like level of hormone in blood plasma, was 0.917, 0.917, and 0.970% respectively, and the best level for the feed conversion ratio was estimated at 0.908 %. Yuan *et al*., (2015) have shown that levels higher than the recommended of L-Arg increased the liver protein synthesis rate and protein gain rate of laying hens.

Conclusion

The present study has found that the content of digestible Arg in the diet may be helpful to improve the secretion of IGF-1, feed conversion ratio, egg production, egg mass, and yolk color of laying hens from 40 to 46 weeks of age. The quadratic equations analysis showed that the optimum levels of digestible Arg for improvement of egg mass, egg production percentage, plasma IGF-1, and feed conversion ratio, were 0.917, 0.917, 0.970, and 0.908 % respectively.

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