

# Effects of dietary supplementation of arginine and lysine on performance and egg quality characteristics of laying quails

Tuba BÜLBÜL<sup>1</sup>, Elmas ULUTAŞ<sup>2</sup>, Mustafa EVCİMEN<sup>2</sup>

University of Afyonkocatepe, Faculty of Veterinary Medicine, <sup>1</sup>Department of Animal Nutrition and Nutritional Disease, <sup>2</sup>Department of Physiology, Afyonkarahisar. Turkey.

**Summary:** The aim of the present research was to determine the effects of arginine and lysine supplementation in laying quail diets on performance and egg quality characteristics. A total of 420 Japanese quails (280 females and 140 males; *Coturnix coturnix japonica*) aged 10 weeks old were divided into seven equal groups, each consisting of 60 quails. The control group was fed a basal diet without the addition of arginine or lysine (with an arginine:lysine ratio at the required level). Experimental groups were fed the basal diet supplemented with: 0.1% arginine; 0.2% arginine; 0.1% lysine; 0.2% lysine; 0.1% arginine + 0.1% lysine; or 0.2% arginine + 0.2% lysine. Feed intake decreased in the 0.1% lysine, 0.2% lysine, and 0.1% arginine + 0.1% lysine groups compared with the control and arginine groups ( $P<0.01$ ). At the end of the experiment, body weight increased for females in all experimental groups ( $P<0.05$ ); eggshell thickness decreased in the 0.2% arginine group ( $P<0.01$ ); and the albumen index increased in the 0.2% arginine + 0.2% lysine group compared with the 0.1% arginine, 0.2% lysine, and 0.1% arginine + 0.1% lysine groups ( $P<0.05$ ). There were no differences between the experimental groups in terms of egg production, egg weight, feed conversion ratio, shape index, Haugh unit, yolk index, albumen index and yolk color index. In conclusion, lysine supplementation in the quail diets did not appear to have any significant negative effect; excessive arginine supplementation might impair the quality of the eggshell; and combined arginine and lysine supplementation did not improve performance.

Key words: Arginine, egg quality characteristics, laying quails, lysine, performance.

## Yumurtacı bıldırcın rasyonlarına arjinin ve lizin ilavesinin performans ve yumurta kalite özellikleri üzerine etkisi

**Özet:** Bu araştırma yumurtacı bıldırcın rasyonlarına arjinin ve lizin ilavesinin performans ve bazı yumurta kalite özellikleri üzerine etkisini belirlemek amacıyla yapıldı. Araştırmada toplam 420 adet (280 dişi ve 140 erkek) 10 haftalık Japon bıldırcını her biri 60 bıldırcından oluşan 7 gruba ayrıldı. Kontrol grubu arjinin ve lizin ilavesi yapılmayan (arjinin:lizin oranı gereksinim düzeyinde olan) temel rasyonla beslenirken, deneme grupları temel rasyona % 0.1 arjinin, % 0.2 arjinin, % 0.1 lizin, % 0.2 lizin, % 0.1 arjinin + % 0.1 lizin ve % 0.2 arjinin + % 0.2 lizin katılarak oluşturuldu. Yem tüketiminin % 0.1 lizin, % 0.2 lizin ve % 0.1 arjinin + % 0.1 lizin gruplarında kontrol ve arjinin gruplarına göre düştüğü belirlendi ( $P<0.01$ ). Deneme sonunda canlı ağırlığın dişilerde tüm deneme gruplarında arttığı ( $P<0.05$ ), yumurta kabuk kalınlığının % 0.2 arjininli grupta azaldığı ( $P<0.01$ ), albumin indeksi ise % 0.2 arjinin + % 0.2 lizin grubunda %0.1 arjinin, %0.2 lizin ve %0.1 arjinin + % 0.1 lizin gruplarına göre arttığı ( $P<0.05$ ) tespit edildi. Yumurta verimi, yumurta ağırlığı, yemden yararlanma oranı, şekil indeksi, Haugh birimi, sarı indeks, ak indeks ve sarı renk indeksi yönünden ise deneme gruplarında değişiklik oluşmadığı belirlendi. Sonuç olarak bıldırcın rasyonlarına lizin ilavesinin belirgin olumsuz etkisi bulunmadığı, gereğinden fazla arjinin ilavesinin yumurta kabuğu kalitesini bozabileceği, arjinin ve lizin birlikte ilavesinin ise performans üzerine etkili olmadığı ifade edilebilir.

Anahtar sözcükler: Arjinin, lizin, performans, yumurta kalite özellikleri, yumurtacı bıldırcın.

## Introduction

In poultry diets based on corn–soybean meal, lysine is specified as the second limiting amino acid, followed by arginine (25). Arginine and lysine are important for poultry nutrition in terms of the promotion and development of growth (8, 11, 12, 19), egg production and egg quality (15, 26, 27, 28). Body-weight (BW) gain, feed intake (FI) and feed conversion ratio (FCR) in poultry are adversely affected by deficiencies of these

amino acids (6, 14, 20, 21). In contrast, excessive levels of these amino acids in the diet have been found to result in impaired growth performance (4, 7, 8, 9, 10), and to not have any positive effects on egg production and quality (2).

It has been reported that the optimum arginine:lysine ratio in a diet enhances the requirement for arginine in association with increasing the lysine level (34). Increasing arginine:lysine ratios have no impact on

livability performance (35), egg production, or weight and quality (31) in the case of adequate levels of both amino acids in the diet. However, Kidd and Kerr (18) determined that an increased arginine:lysine ratio in the diet positively affects BW gain and FCR. Therefore, the objective of this study was to determine the effects of arginine, with or without lysine supplementation, at different levels in Japanese quail diets on performance and egg quality characteristics.

### Materials and Methods

**Animals:** A total of 420 (280 females and 140 males) 10-week-old Japanese quails (*Coturnix coturnix japonica*) were used in this study. The research procedure was approved by the Ethics Committee of Afyon Kocatepe University (AKÜHADYK-214-13). There were seven groups, each containing 60 laying quails, in the experiment. Each group was divided into four replicate groups consisting of 15 quails each. Quails were placed in individual pens for 8 weeks and were given feed and water *ad libitum* under conditions of 16 hours light–8 hours dark.

**Diets and experimental treatments:** The feed raw materials were obtained from a commercial company (Tinaztepe Feed Factory, Afyonkarahisar). The basal diet (20.00% crude protein, 1.24:0.99 arginine:lysine ratio, and 2900 kcal/kg metabolizable energy) was formulated to meet the nutrient requirements of quails recommended by the National Research Council (NRC) (25).

The control group was fed a basal diet without the addition of arginine or lysine (Table 1). The experimental groups were fed the basal diets with supplementation of 0.1% arginine (L-arginine, Sigma A5131 powder form); 0.2% arginine; 0.1% lysine (L-lysine-HCl); 0.2% lysine; 0.1% arginine + 0.1% lysine; or 0.2% arginine + 0.2% lysine, respectively. The nutrient composition of the basal diet, including moisture, crude protein, crude fat, crude fibre, crude ash and calcium contents, was determined according to the AOAC (1). The metabolizable energy level of the basal diet was calculated according to Carpenter and Clegg (23). The arginine and lysine levels of the basal diet were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Applied Biosystems API-3200; ANT Technical Devices Laboratory) (Table 1).

**Performance parameters:** BW was determined at the beginning and end of the study. FI was identified biweekly as the group average. FCR was calculated on the same days as the amount of feed consumed per kg egg. Daily egg production records were kept in groups. The eggs' weights were measured biweekly.

**Egg quality parameters:** In this study, 12 eggs from each group (three eggs from each replicate) were collected to determine the internal and external quality parameters of the eggs once every 4 weeks. The eggs were

Table 1. Ingredients and chemical composition of basal diet, %.  
Tablo 1. Temel rasyonun yem ham maddeleri ve besin madde bileşimi, %.

Ingredients	%
Corn	50.02
Wheat	3.96
Full fat soybean	12.07
Soybean meal	14.04
Sunflower meal	3.59
Corn gluten meal	6.66
Vegetable oil	2.00
Limestone	5.05
Salt	0.33
Dicalcium phosphate	1.55
NaHCO <sub>3</sub>	0.23
L-Lysine	0.10
DL-Methionine	0.05
Vitamin mineral premix <sup>1</sup>	0.35
Chemical composition (analysed)	
Dry matter, %	91.35
Crude ash, %	9.42
Crude protein, %	20.60
Ether extract, %	6.04
Crude fiber, %	3.52
Nitrogen free extract, %	51.77
Calcium, %	2.50
Available phosphorus, %	0.35
Metabolisable energy <sup>2</sup> , kcal kg <sup>-1</sup>	2914
Arginine <sup>3</sup> , %	1.24
Lysine <sup>3</sup> , %	0.99
Methionine <sup>4</sup> , %	0.45
Methionine+cystine <sup>4</sup> , %	0.81

<sup>1</sup> Each kg of diet: Retinol acetate, 4.1 mg; cholecalciferol, 0.075 mg; DL- $\alpha$ -tocopherylacetate, 11 mg; menadione, 1 mg; thiamin, 2.8 mg; riboflavin sodium phosphate, 5.8 mg; niacin, 44 mg; pyridoxine, 4 mg; cyanocobalamin, 0.026 mg; Ca-D-pantothenate, 8.8 mg; folic acid, 1 mg; choline, 220; biotin, 0.11 mg; Cu, 6 mg; I, 1.1 mg; Fe, 30 mg; Se, 0.1 mg; Mn, 60 mg; Zn, 50 mg

<sup>2</sup> Metabolisable energy (ME) content of diets was estimated using the equation devised by Carpenter and Clegg (Leeson and Summers, 2001):  
ME (kcal/kg) = 53 + 38 [(crude protein %) + (2.25 x ether extract %) + (1.1 x starch %) + (sugar %)].

<sup>3</sup> The arginine and lysine levels of the basal diet were determined by LC-MS-MS (Applied Biosystems API-3200, ANT Technical Devices Lab.)

<sup>4</sup> The methionine and methionine+cystine levels of the basal diet were calculated according to the Batal and Dale (3)

examined for weight (g), length (mm), width (mm), eggshell thickness (mm), fracture strength, albumen index (%), yolk index (%), Haugh unit, egg shape index and yolk color index. Egg width, egg length, yolk width, albumen length and albumen width were measured by compass to the nearest 0.01 mm. Albumen and yolk heights were measured by micrometer to the nearest 0.01 mm. Egg shape, yolk and albumen indexes were calculated from these measures (5). The Haugh unit was calculated with the formula developed by Haugh (16). Measurements of the thickness of dried shells with the membrane were obtained from two sides in the equatorial region, as well as on the blunt and pointed edges with a micrometer to the nearest 0.01 mm (5). The egg yolk visual color score was determined by matching the yolk with one of the 15 bands of the 1961 'Roche improved yolk color fan'. The formulas used in the measurement of egg quality parameters were as follows:

$$\text{Shape index (\%)} = [\text{egg width (mm)}/\text{egg length (mm)}] \times 100$$

$$\text{Yolk index (\%)} = [\text{yolk height (mm)}/\text{yolk width (mm)}] \times 100$$

$$\text{Albumen index (\%)} = \text{albumen height (mm)} / [(\text{average albumen length (mm)} + \text{width (mm)})/2] \times 100$$

$$\text{Haugh unit} = 100 \log [\text{albumen height (mm)} + 7.57 - 1.7 \times \text{egg weight}^{0.37} \text{ (g)}]$$

*Statistical analysis:* The variance analysis method was used for all statistical calculations and the significance of differences between the mean values of the groups according to the data obtained from the research. Duncan's test was applied to control the significant difference between groups (SPSS 10.00; SPSS Inc., Chicago, IL, USA). A value of  $P < 0.05$  was considered as the limit for statistical significance (13).

## Results

*Performance:* Initial BW did not change depending on sex in the groups in which arginine and lysine were given singly or combined ( $P > 0.05$ ). Compared with the final BW of female and male quails, arginine and lysine supplementation did not cause a change in the BW of males, whereas the BW of females increased ( $P < 0.05$ ) in all experimental groups compared with the control group. FI did not change over the course of the experiment ( $P > 0.05$ ), whereas it was determined during the entire research that FI reduced ( $P < 0.01$ ) in the 0.1% lysine, 0.2% lysine, and 0.1% arginine + 0.1% lysine groups compared with the control and arginine groups. The values of egg production, egg weight and FCR were not affected by arginine and lysine levels in the diets in the experiment (Table 2).

*Egg quality:* Eggshell thickness and the albumen index showed no changes in any of the experimental groups compared with the control group in the 4th week.

In the 8th week, eggshell thickness decreased ( $P < 0.01$ ) in the 0.2% arginine group compared with the control and other experimental groups. Albumen index increased ( $P < 0.05$ ) in the 0.2% arginine + 0.2% lysine group compared with the 0.1% arginine, 0.2% lysine, and 0.1% arginine + 0.1% lysine groups. At the 4th and 8th weeks of the experiment no changes in the other egg quality parameters were determined in terms of the shape index, yolk index, Haugh unit and yolk color index (Table 3).

## Discussion and Conclusions

In the present study, the control group diet was formulated to have optimal arginine and lysine levels and arginine:lysine ratio according to NRC (25) recommendations; the ratio of arginine:lysine was found to be 1.24:0.99. Final BW increased in female quails ( $P < 0.05$ ; Table 2). Several studies conducted with broilers (6, 19, 21, 34) reported that arginine and lysine supplementation affected BW gain positively, while it was decreased by arginine and lysine deficiency (14, 20, 22). On the other hand, excessive arginine and lysine supplementation in the diet (twice the level of requirement) has been reported to lower BW gain for chicks (7) or keep it steady for chickens (2) and chicks (4, 32). Other studies reported that arginine and lysine supplementation had positive effects only under stressful circumstances (33); the requirement changed depending on the protein level of the diet (17, 24). In the present study, the BW of female quails increased due to arginine and lysine supplementation; the species and sex of the poultry could be a factor when considering the supplementation of amino acids in diets. In addition, the diets suggested for chickens might not always be valid for quails.

During the experiment, total FI decreased ( $P < 0.01$ ) in the lysine-only-supplemented groups and the 0.1% arginine + 0.1% lysine group (Table 2). In laying hens, supplementation with arginine and lysine in combination at the same levels (2) and an increase of lysine only in the diet have been reported to be probable causes of FI reduction (30). Also, it has been reported that high lysine levels in the diets of broilers might reduce FI (7, 24). Some studies (6, 20) suggest that arginine and lysine deficiency in diets might have a suppressive effect on FI. Nevertheless, Basiouni et al. (2) reported that increasing levels of lysine, more than arginine, in laying hens increased FI when compared with other groups where arginine and lysine were increased together. Contrary to our results, it has also been recorded that 0.15% lysine supplementation in the diet (28), increased lysine levels in the diets (15, 26), and an enrichment of a diet with lysine (36) does not affect FI. Moreover, it has been reported that different arginine:lysine ratios (1.16, 1.21, 1.26, 1.31 and 1.36) (31), by changing the arginine and lysine levels in laying quail diets, and the

Table 2. The effects of arginine and lysine levels on performance of quail  
 Tablo 2. Arjinin ve lizin düzeylerinin bıldırcınlarda performans üzerine etkisi.

	Control	0.1% Arginine	0.2% Arginine	0.1% Lysine	0.2% Lysine	0.1% Arginine + 0.1% Lysine	0.2% Arginine + 0.2% Lysine	SEM	P
Initial body weight, g									
Female	196.76	197.54	196.28	198.22	201.20	198.54	197.78	1.45	0.987
Male	181.12	180.16	183.45	177.64	177.80	181.50	178.24	1.79	0.943
Final body weight, g									
Female	205.14b	223.76a	216.67a	220.06a	226.13a	223.61a	219.10a	1.60	0.016
Male	195.40	201.42	205.90	198.30	198.40	209.11	207.12	1.41	0.073
Egg production, %									
2nd week	80.00	74.20	82.20	73.80	74.60	73.20	71.50	1.41	0.397
4th week	80.71	80.35	81.25	86.25	81.07	83.21	77.67	1.24	0.728
6th week	77.22	81.38	76.11	83.05	85.83	83.33	79.16	1.41	0.534
8th week	78.28	79.37	73.59	81.09	80.31	79.37	81.71	1.15	0.622
0-8 weeks	79.00	78.83	78.83	81.04	80.45	79.78	77.83	0.68	0.907
Egg weight, g									
2nd week	12.04	12.20	12.07	11.88	12.34	12.53	12.60	0.09	0.301
4th week	12.76	13.14	13.00	13.25	13.40	13.49	12.69	0.09	0.156
6th week	12.91	12.97	12.22	12.69	12.91	13.16	12.43	0.10	0.157
8th week	13.59	13.73	13.17	13.29	13.56	13.67	13.14	0.11	0.721
0-8 weeks	12.92	13.01	12.61	12.72	13.05	13.21	0.06	0.09	0.234
Feed intake, g/day									
2nd week	26.92	25.90	26.11	25.70	25.29	25.09	24.88	0.28	0.563
4th week	26.72	27.54	27.13	25.50	26.11	26.92	28.35	0.27	0.104
6th week	27.94	28.15	28.56	26.92	26.31	26.11	26.52	0.28	0.111
8th week	28.76	29.17	29.58	27.13	26.52	27.74	27.94	0.29	0.049
0-8 weeks	27.59a	27.69a	27.84a	26.31b	26.06b	26.46b	26.92ab	0.16	0.001
Feed conversion ratio, kg feed /kg egg									
2nd week	3.02	3.36	2.93	3.49	3.18	3.28	3.73	0.10	0.470
4th week	2.60	2.61	2.60	2.28	2.41	2.41	2.91	0.05	0.122
6th week	2.75	2.68	3.08	2.57	2.38	2.38	2.75	0.06	0.038
8th week	2.74	2.76	3.06	2.48	2.45	2.57	2.60	0.06	0.191
0-8 weeks	2.72	2.81	2.90	2.72	2.56	2.59	2.92	0.04	0.238

Letters (a, b) in the same line indicate significant differences between different letters.

supplementation of 0.25% lysine (29) do not change FI. The decrease in FI in the groups in which only lysine was supplemented in the present study might have stemmed from inadequate arginine levels in the diet, as Kwak et al. (21) reported. The FI decrease in groups with low levels of arginine and lysine supplementation might be linked to the birds lowering the FI in order to protect the arginine:lysine ratio, even though arginine and lysine increased together.

In the present study, arginine and/or lysine supplementation in diets resulted in no differences between the groups in terms of egg production, egg weight and FCR during the separate weeks or the entire study (Table 2). It has been reported that supplementation of arginine and lysine in the diet does not affect egg production, egg weight or FCR in laying quails (31) and hens (2). In contrast, it has also been reported that 0.25%

lysine supplementation in quail diets reduced egg weight (29) and 0.15% lysine supplementation increased egg production and egg weight in hens (28). The present research shows that arginine and lysine do not affect egg production, egg weight and FCR of quails at the applied levels.

In this study, no changes were recorded in the 4th and the 8th weeks for egg quality parameters such as shape index, yolk index, Haugh unit and yolk color. In the 4th week no changes between the groups were determined for albumen index (Table 3). Basiouni et al. (2) also reported that arginine:lysine ratios from the contribution of arginine and lysine at different levels into the diet do not affect albumen height, which is an internal egg quality characteristic. In contrast, the arginine-forming arginine silicate–inositol complex was claimed to increase the Haugh unit (27). Eggshell thickness was

negatively affected by the high level of arginine supplied in the 8th week ( $P < 0.01$ , Table 3). Contrary to our findings, Onderci et al. (27) reported that due to the relationship between silicon and calcium (which is vital for determining shell thickness), and arginine's disruption of the absorption of calcium, dietary arginine silicate–inositol complex supplementation at 0.05% and 0.1% levels led to an increase in shell thickness. Conversely, Önel et al. (28) found that 0.15% lysine supplementation in hens did not change the shell thickness. The decrease in the thickness of the shell in this study might have resulted from the arginine:lysine ratio being much higher in the group in which arginine was used at an excessive level compared with the other groups. In the 8th week of the research the albumen index increased ( $P < 0.05$ ) in the 0.2% arginine + 0.2% lysine group compared with the low level of arginine and arginine + lysine groups, as well as the high-level lysine groups (Table 3). This increase in the albumen index could be linked to the use of arginine and lysine together at high levels and an increased level of protein. Moreover, the high level of the albumen index in the 0.2% arginine + 0.2% lysine group might have resulted from the egg weights being quantitatively higher in this group than in the others.

As a result, arginine supplementation (0.1% and 0.2%) with or without lysine supplementation (0.1% and 0.2%) in the diets of quails did not affect egg production, egg weight or FCR; however, arginine supplementation at the level of 0.2% reduced eggshell thickness. Hence, arginine and/or lysine supplementation above the required levels in quail diets may not lead to a change in performance and egg quality. The arginine and lysine levels reported by the NRC may be sufficient for feeding laying quails. Moreover, it is suggested that studies are needed to clarify the relationship of the arginine:lysine ratio with different environmental conditions, electrolyte balance, or other amino acids.

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Table 3. The effects of arginine and lysine levels on egg quality parameters of quail

Tablo 3. Arjinin ve lizin düzeylerinin bıldırcınlarda yumurta kalite parametreleri üzerine etkisi.

	Control	0.1% Arginine	0.2% Arginine	0.1% Lysine	0.2% Lysine	0.1% Arginine + 0.1% Lysine	0.2% Arginine + 0.2% Lysine	SEM	P
<b>Egg shell thickness, mm/100</b>									
4. hafta	20.23	20.04	20.00	18.86	18.86	19.78	19.90	0.21	0.407
8. hafta	20.11a	18.71a	17.03b	19.24a	18.83a	19.50a	20.04a	0.22	0.002
<b>Haugh unit<sup>1</sup></b>									
4. hafta	82.79	79.48	80.06	82.57	79.72	78.23	86.08	1.31	0.736
8. hafta	79.83	75.60	78.75	81.25	76.29	79.58	85.53	1.38	0.480
<b>Shape index<sup>2</sup>, %</b>									
4. hafta	77.50	76.96	77.32	79.03	76.27	77.78	78.87	0.34	0.226
8. hafta	80.71	78.51	78.78	80.18	78.57	79.76	80.80	0.32	0.316
<b>Yolk index<sup>3</sup>, %</b>									
4. hafta	42.62	42.32	42.52	43.90	41.94	42.77	43.37	0.17	0.226
8. hafta	43.31	42.10	42.25	43.02	42.14	42.80	43.36	0.18	0.326
<b>Albumen index<sup>4</sup>, %</b>									
4. hafta	8.56	8.09	8.27	8.38	7.74	8.10	9.49	0.15	0.067
8. hafta	8.61ab	7.85b	8.28ab	8.39ab	7.37b	8.12b	9.34a	0.15	0.026
<b>Yolk colour index</b>									
4. hafta	7.91	7.60	8.06	8.13	7.80	8.33	7.73	0.11	0.636
8. hafta	7.16	7.26	7.46	7.86	7.68	8.03	7.58	0.11	0.156

<sup>1</sup> Haugh unit =  $100 \log [\text{albumen height (mm)} + 7.57 - 1.7 \times \text{egg weight}^{0.37} \text{ (g)}]$

<sup>2</sup> Shape index (%) =  $[\text{egg width (mm)} / \text{egg length (mm)}] \times 100$

<sup>3</sup> Yolk index (%) =  $[\text{yolk height (mm)} / \text{yolk width (mm)}] \times 100$

<sup>4</sup> Albumen index (%) =  $\text{albumen height (mm)} / [(\text{average albumen length (mm)} + \text{width (mm)}) / 2] \times 100$

Letters (a, b) in the same line indicate significant differences between different letters.

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**Address for correspondence:**

Dr. Tuba Bülbül  
University of Afyonkocatepe,  
Faculty of Veterinary Medicine,  
Department of Animal Nutrition and Nutritional Disease,  
ANS Campus, Afyonkarahisar, Turkey,  
e-mail: tbulbul@aku.edu.tr