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Original Article

■Author(s)

Kheiri F^I Landy N^I b https://orcid.org/0000-0001-5517-4524

¹ Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

■Mail Address

Corresponding author e-mail address Farshid Kheiri Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Phone: +98 913 1114 9172 Email: farshid_kheiri@yahoo.com

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ABSTRACT

This experiment was conducted to examine the effects of dietary L-arginine (Arg) supplementation on growth performance, intestinal morphology, serum biochemical and hematological parameters in meat-type quails. A total of one hundred and sixty 7-d-old male quail chicks were weighed and allocated to four treatment groups of four replicates, with 10 chicks/replicate. Four groups of quail chicks were fed basal diet containing 12.44 g lysine (Lys)/kg of diet (control), basal diet supplemented with 0.4, 1.21 and 1.84 g Arg/kg of diet (for Arg to Lys ratios of 100, 103, 109 and 114) for 28 days. The birds were fed diets from 7 to 38 d of age. Final body weight and feed:gain tended to improve by increasing Arg:Lys ratio to 109. Relative weight of spleen and bursa were significantly (p < 0.05) increased by the addition of 0.4 g Arg/kg of diet. Morphometric parameters of jejunum were not affected by the dietary treatments. The uric acid, and triglyceride concentration of the serum significantly (p<0.05) decreased by increasing the Arg:Lys ratio to 114. Serum HDL-cholesterol concentration were significantly (p<0.05) increased by increasing the Arg:Lys ratio to 114. Monocytes were decreased by increasing the Arg:Lys ratio to 114. In conclusion, our results indicated that Arg supplementation in the quail diets did not appear to have any significant positive effects on performance criteria and although increasing Arg:Lys ratio to 114 could improve serum biochemical parameters.

INTRODUCTION

Nutrient requirements of Japanese quail (*Coturnix coturnix japonica*) and bobwhite quail (*Colinus virginianus*) have been specified by NRC, (1994). Other than the nutrient requirements suggested by NRC (1994), a few investigations have been done to update nutrition specifications for quails (Silva & Costa 2009; Rostagno *et al.*, 2011; Rostagno *et al.*, 2017). This is despite the fact that nowadays quails are heavier and more productive due to genetic evolution.

Arginine (Arg), a basic amino acid, is an essential amino acid (AA) for poultry because bird's enterocytes are unable to make Arg from endogenous sources (Boorman & Lewis 1971). Arg serves a number of physiological and biological functions in poultry because it's a substrate for biosynthesis of many molecules, including protein, polyamines, biological effector molecule nitric oxide, creatine, ornithine, glutamate, proline, glutamine, agmatine and dimethylargininesa (Beaumier *et al.*, 1996; Efron & Barbul 1998, 2000; Khajali & Wideman 2010). As a result, Arg requirements of poultry represent and warrant the metabolic demand for protein and metabolite synthesis (Ball *et al.*, 2007).

Amino Acid/Lysine (Lys) ratios are used to estimate AA requirements of poultry. In several trials in poultry researchers evaluated efficacy of



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higher AA/Lys ratios in comparison with recommended ratios. Esser *et al.* (2017) investigated efficacy of Arg supplementation on the carcass yield and meat quality of broilers subjected to two days of heat stress before slaughter; the results indicated that Arg supplementation to broiler chicken diet resulted in heavier carcasses, higher breast yield, and lower of abdominal fat deposition compared with the control group. Reis *et al.* (2012) investigated the efficacy of different Arg:Lys ratios (1.16, 1.21, 1.26, 1.31 and 1.36) on performance and egg quality of laying Japanese quails; the results showed that the best ratio for laying Japanese quails is 1.16. Bülbül *et al.* (2015) reported that increasing Arg:Lys ratio in laying Japanese quails impaired the quality of the eggshell.

Despite wide knowledge about using Arg in poultry diet, to date no tests have been conducted to examine probable efficacy of different Arg:Lys ratios in meattype quails; thus the present trial was carried out to examine the effects of dietary Arg supplementation on growth performance, intestinal morphology, serum biochemical and hematological parameters in meattype quails (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

Animals and dietary treatments

All procedures in the present trial were approved by the ethical committee for experimental use of birds of the Shahrekord University farm under accession number Q11/2017. This study was carried out to examine the effects of dietary L-arginine (Arg) supplementation on growth performance, lymphoid organs weight, intestinal morphology, serum biochemical and hematological parameters in meat-type quails. A total of one hundred and sixty 7-d-old male quail chicks (Coturnix japonica) were weighed (17±1) and allocated to four treatment groups of four replicates, with 10 chicks/replicate. Four groups of quail chicks were fed basal diet containing 12.44 g Lys/kg of diet (control), basal diet supplemented with 0.4, 1.21 and 1.84 g Arg/kg of diet (for Arg to Lys ratios of 100, 103, 109 and 114) for 28 days. The birds were fed diets from 7 to 38 d of age. Basal diet was formulated to meet nutrient requirement of Japanese quail recommended by Rostagno (2011). Prior to formulating the diets, corn and soybean meal were subjected to crude protein (CP) and total amino acid (AA) analyses. CP and AA composition of the formulated diets including Arg and Lys were measured after acid hydrolysis (AOAC International, 2006). Meat-type quails had

free access to feed and water throughout the trial period. The quails were housed in wire cages (106 cm²/ quail) and room temperature was maintained at 33°C at the arrival of the chicks, and gradually decreased by 2.5 °C/wk. The lighting was furnished by incandescent bulbs to provide continuous lighting.

Growth performance and lymphoid organs weight

Body weights (BW) by replicate were assayed on the 7th day and on the termination of the trial (35th day). Average daily weight gain (DWG), average daily feed intake (DFI), and feed to gain ratio were calculated on the 35th day. Mortalities were recorded to correct DFI. At d 35, 2 quails in each replicate were randomly chosen based on the average BW of the replicate, euthanized by cutting the carotid artery of the neck and bursa and spleen were removed, weighed and calculated as a percentage of live body weight.

Morphology of the Jejunum

On the 35th day of age, a cm of jejunum from 2 quais/replicate were removed from the midpoint section, rinsed in 4% formalin-buffered saline solution. The samples were processed, paraffin embedded and histological examination was carried out according to the method described by Kavyani *et al.* (2014).

Serum biochemical and hematological parameters

On the 35th day of age, 2 quails per replicate were chosen and blood samples were taken from brachial vein and samples were divided into heparinised and non-heparinised tubes. The samples from tube without anticoagulant were centrifuged to obtain serum. The serum samples were analyzed for total protein, albumin, urea, uric acid, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol and triglyceride (Kit package, Pars zmoon Company; Tehran, Iran). The serum globulin concentrations of quails were computed by subtracting albumin concentration from proteins.

The samples from non-heparinised tube were used to prepare blood smears using May-Greenwald-Giemsa stain. One hundred leukocytes per samples were counted under an optical microscope (100 x oil immersion). The percentage of hematocrit or packed cell volume (PCV) was measured by microhematocrit method (Kececi *et al.*, 1998). Also total white blood cell (WBC) counts were determined by brilliant cresyl blue dye (Haddad & Mashaly, 1990).



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Statistical analysis

The experiment was carried out as a completely randomized design, and all obtained data were analyzed using the statistical software package SAS University (SAS, 2012). Means were compared using Tukey test at 5% significance.

RESULTS AND DISCUSSION

Growth performance and lymphoid organs weight

The calculated and analyzed compositions of dietary treatments are shown in Table 1. The analyzed

Table 1 – Composition of the dietary treatments.¹

Ingredient, g/kg	Arginine to lysine ratio					
	100	103	109	114		
Corn	620.77	623.03	628.25	634.44		
Soybean meal, 44% CP	309	308	307	305		
Starch	10.0	9.5	6.8	3.9		
Soybean oil	5.0	5.0	5.0	5.0		
DL-Methionine	2.35	2.35	2.34	2.34		
L-Lysine	3.38	3.39	3.43	3.46		
L-Threonine	1.81	1.82	1.83	1.84		
L-Valine	1.41	1.41	1.42	1.43		
L-Arginine	0.00	0.40	1.21	1.84		
L-Isoleucine	0.60	0.60	0.62	0.64		
L-Glutamic acid	8.00	6.80	4.40	2.40		
Choline Chloride 60%	1.80	1.80	1.80	1.80		
Dicalcium Phosphate (22 Ca, 17 P)	16.93	16.93	16.92	16.90		
Calcium Carbonate	11.83	11.84	11.85	11.87		
Sodium Bicarbonate	3.79	3.80	3.82	3.83		
Common Salt	1.33	1.33	1.31	1.31		
Trace mineral premix ¹	1.0	1.0	1.0	1.0		
Vitamin premix ²	1.0	1.0	1.0	1.0		
Calculated composition						
Metabolizable energy, kcal/kg	2,919	2,919	2,919	2,919		
Crude protein, g/kg	205.6	205.6	205.6	205.6		
Lysine, g/kg	12.44	12.44	12.44	12.44		
Methionine, g/kg	5.29	5.29	5.29	5.29		
Methionine+cysteine, g/kg	8.44	8.44	8.44	8.44		
Threonine, g/kg	9.15	9.15	9.15	9.15		
Tryptophan, g/kg	2.36	2.36	2.36	2.36		
Arginine, g/kg	12.45	12.83	13.60	14.20		
Isoleucine, g/kg	8.98	8.98	8.98	8.98		
Leucine, g/kg	16.9	16.9	16.9	16.9		
Valine, g/kg	10.68	10.68	10.68	10.68		
Calcium, g/kg	9.0	9.0	9.0	9.0		
Nonphytate P, g/kg	3.75	3.75	3.75	3.75		
Sodium, g/kg	1.76	1.76	1.76	1.76		
Chlorine, g/kg	2.03	2.03	2.03	2.03		
Potassium, g/kg	7.94	7.92	7.92	7.90		
DCAB	222.0	221.8	221.8	221.3		
Analyzed Content						
Crude protein, g/kg	20.8	20.7	20.6	20.7		
Lysine, g/kg	12.6	12.4	12.5	12.5		
Arginie, g/kg	12.7	12.9	13.5	14.5		
Arginine: Lysine	100	104	108	116		

¹Provided the following per kg of diet: Mg, 60 mg; Fe, 120 mg; Cu, 5 mg; Zn, 25 mg; Se, 0.2 mg; I, 0.3 mg.

²Provided the following per kg of diet:vitamin A, 1,650 IU; vitamin E, 12 IU; vitamin K, 1 mg; Thiamin, 2 mg; Riboflavin, 4 mg; vitamin B₆, 3; vitamin B₁₂, 0.003 mg; pantothenic acid, 10 mg; nicotinic acid, 40 mg; folic acid, 1 mg; Biotin, 0.3 mg.

DCAB: Dietary cation anion balance



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content of diets including CP, Arg, Lys, and Arg:Lys ratios are in close agreement with the calculated composition. Performance data of meat-type quails fed different levels of L-Arg are shown in Table 2. The supplementation of different levels of Arg to basal diet did not markedly affect final BW, DFI, and FCR of meat-type quails, although final BW and FCR tended to improve by increasing Arg:Lys ratio to 109 (p>0.05). Atencio et al. (2004) reported that supplementation of different levels of Arg to male broiler chickens' diet couldn't affect performance criteria. Reis et al. (2012) investigated the efficacy of different Arg:Lys ratios on performance and egg guality of Japanese quails; the result indicated that laying Japanese quails didn't respond to increasing Arg:Lys ratios. Bülbül et al. (2015) reported that the addition of different levels of Arg to laying Japanese guails' diet couldn't improve performance parameters. Maurício et al. (2016) investigated the efficacy of supplementing different inclusion rates of Arg (Arg to Lys ratios of 1.06, 1.16, 1.26, 1.36, and 1.46) on egg production of laying Japanese quails; the researchers' report indicated that the performance of laying Japanese quails didn't respond to increasing Arg:Lys ratio. Brake et al. (1998), reported that increasing Arg:Lys

ratio in broiler chickens' diet raised under heat stress condition improved FCR of broilers. Veldkamp *et al.* (2000) studied the effects of Arg:Lys ratio on growth performance of male turkeys raised under heat stress condition; the researchers' report indicated that increasing Arg:Lys ratio resulted in heavier BW at 98 d of age and it may be due to the higher DFI.

In the present trial meat-type quails didn't respond to increasing Arg:Lys ratio, although final BW and FCR tended to improve by increasing Arg:Lys ratio to 109. An explanation for the lack of an Arg:Lys effect may be the presence of stress in the trial. It seems that the presence of a stress such as high or low temperature is necessary to observe significant effects of Arg on performance parameters while the present trial was carried out in optimum conditions. Another explanation is the level of Lys which we have used in the present trial; as reported by Veldkamp et al. (2000) dietary Arg content is important when dietary Lys is marginal; while in the present trial adequate level of Lys was provided in the dietary treatment. More investigations are required to clarify the effects of increasing Arg:Lys ratio in meat-type quails reared under heat stress condition.

Table 2 – Effect of the dietar	ry treatments on the growth	performance of the Japanese	quails (Coturnix iaponica)
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Variable1		Arginine to lysine ratio				
Variable ¹ —	100	103	109	114	SEM	p-value
Body weight, g	207.7	207.5	212.2	208.2	4.10	0.82
Daily feed intake, g/d	17.2	17.0	17.2	17.3	0.23	0.82
Daily weight gain, g/d	6.8	6.8	7.0	6.8	0.14	0.83
FCR, g:g	2.49	2.47	2.44	2.51	0.05	0.79

Values in the same row not sharing a common superscript differ significantly (p<0.05).¹FCR = feed:gain ratio; SEM = standard error of mean.

The relative weights of lymphoid organs are shown in Table 3. Relative weight of the spleen was significantly (p<0.05) increased by the addition of 0.4 g Arg/kg of the diet (0.15 %), compared with those fed the basal diet (0.10 %). Meat-type quails fed 0.4 g Arg/kg of the diet had significantly higher (p<0.05) relative weight of bursa compared to the other groups. Similarly, Murakami *et al.* (2014) reported that the addition of Arg in broiler chickens' diet resulted in

greater weight and dimensions of the bursa compared to nonsupplemented broilers. Kwak *et al.* (1999) reported that broiler chicks fed Arg deficiency diets had lower weight of thymus as a percentage of live BW. In the same study, researchers report indicated that Arg supplementation could increase relative weight of spleen; although Arg supplementation failed to induce any marked effects on the relative weight of the bursa (Kwak *et al.*, 1999). Similar to ours,

Table 3 – The effect of dietar	v treatments on relative	weight of lvm	phoid organs at d 35.

Lumphoid ergans		Arginine	_			
Lymphoid organs	100	103	109	114	SEM	p-value
Bursa*	0.25 ^b	0.33ª	0.23 ^b	0.23 ^b	0.02	0.03
Spleen*	0.10 ^b	0.15ª	0.10 ^{ab}	0.11 ^{ab}	0.01	0.03

^{a-c}Values in the same row not sharing a common superscript differ significantly (p < 0.05).

*Percentage of live body weight.

SEM = standard error of mean.



Toghyani *et al.* (2019) reported that in ovo injection of Arg significantly enhanced weights of the spleen and the bursa as a percentage of live BW on d 11. In contrast with our results Abdukalykova and Ruiz-Feria (2006) reported that the addition of different levels of Arg in broiler chickens diet had no significant effect on the relative weight of the thymus, bursa, and spleen. These data revealed that the development of lymphoid organs is very sensitive to Arg concentration of diet.

Morphometric analysis of the jejunum

Table 4 shows the effects of Arg supplementation on villus height (VH) and width (VW), crypt depth (CD), and villus: crypt ratio (VH/CD) in jejunum of meattype quails at d 35. The supplementation of different levels of Arg to basal diet did not markedly affect (*p*>0.05) morphometric parameters of jejunum at d 35. In contrast to ours, Khajali *et al.* (2014) reported that increasing dietary Arg content of broiler chickens reared at high altitude improved VH, VW and surface area in duodenum and jejunum. Abdulkarimi *et al.* (2019) reported that addition of Arg in broiler chickens challenged with ascites as a consequence of rearing under cold environment reduced CD in duodenum and jejunum, and enhanced ileum VW and also VH/ CD in duodenum and jejunum. Murakami *et al.* (2012) Growth Performance, Intestinal Morphology, Serum Biochemical and Hematological Parameters in Japanese quail (Coturnix japonica) Fed Supplemental L-Arginine

showed that dietary Arg supplementation in broiler chickens' diet improved intestinal mucosa development. Xaio et al. (2016) indicated that the supplementation of Arg in rat exert protective effects against oxidative stress in rat's intestine and also increased VH/CD. As polyamines (putrescine, spermine and spermidine) plays an important role in development of small intestinal and colonic mucosal growth and development and Arg involved in polyamines synthesis (Loser et al., 1999), an improvement in morphometric parameters of jejunum in meat-type quails was anticipated. Although in the present trial the dietary Arg supplementation couldn't induce any marked effect on morphometric related parameters. It is probably due to the optimum environmental condition in the present study. As reported by Landy & kavyani (2013) the environmental stressors such as high or low temperature can disrupt the normal balance of the intestinal microbial ecology and morphology (Landy & kavyani 2013). Mostly, research trials about efficacy of Arg supplementation on gut development are performed in stressful conditions (Khajali et al., 2014; Xaio et al., 2016; Abdulkarimi et al., 2019) and Arg alleviated the adverse effects of environmental stressors; thus maybe our chicks didn't respond to Arg supplementation as a result of optimum environmental condition.

Table 4 – Effects of dietary treatments on villus height and width, crypt depth, and villus height to crypt depth in jejunum at d 35.

Variable		Arginine to lysine ratio				
valiable	100	103	109	114	SEM	p-value
Villus height (µm)	25.1	22.7	23.4	17.4	2.41	0.18
Crypt depth (µm)	46.2	47.0	41.7	40.5	2.86	0.41
Villus width (µm)	17.3	17.8	15.3	13.6	1.87	0.30
Villus height/ Crypt depth	0.55	0.48	0.55	0.42	0.07	0.40

SEM = standard error of mean.

Hematological parameters

The hematological data are presented in Table 5. No differences were observed in the values of packed cell volume and lymphocyte. The means of monocytes values were significantly (p<0.05) lower in meat-type

quails fed diets containing 1.84 g Arg/kg of diet (0.62× $10^{3}/\mu$ L) compared with those fed basal diet (0.82× $10^{3}/\mu$ L), but didn't significantly (*p*>0.05) differ from those fed diets containing 0.4 Arg/kg of diet (0.80× $10^{3}/\mu$ L) or 1.21 g Arg/kg of diet (0.70× $10^{3}/\mu$ L). The eosinophil

Table 5 – Hematological responses of broiler quails fed diets differ in arginine to lysine ratio at d 35.

Parameters		Arginine to lysine ratio				
	100	103	109	114	SEM	p-value
PCV ¹ (%)	47.5	43.2	42.2	41.0	1.55	0.059
WBC ² (× 10 ³ /µL)	19.2 ^{ab}	22.8ª	16.6 ^b	22.3ª	1.65	0.006
Lymphocytes (× 10 ³ /µL)	58.5	62.5	61.5	57.2	2.17	0.340
Monocytes (× 10 ³ /µL)	0.82ª	0.80 ^{ab}	0.70 ^{ab}	0.62 ^b	0.44	0.028
Eosinophils (× 10 ³ /µL)	0.3	0.17	0.12	0.12	0.46	0.06

 $^{1}PCV = packed cell volume; WBC = white blood cell. SEM = standard error of mean.$

^{a,b}values in the same row not sharing a common superscript differ (p < 0.05).



values were not significantly (p>0.05) affected by the dietary treatments. The result of the experiment performed by Perez-Carbajal et al. (2010) showed that the in vitro heterophil and monocyte oxidative burst could be improved in diets with normal levels of vitamin E (VE) with supplementation of intermediate levels of Arg in broiler chickens challenged with coccidiosis. Liu et al. (2014) reported that VE and Arg supplementation in broiler chickens' diet enhanced immune response after a Salmonella Typhimurium challenge. As reported by Li et al. (2002) thymus, spleen and bone marrow are the organs which are related to blood cell formation. Since, in the present experiment the highest relative weight of bursa and spleen obtained in birds supplemented with 0.4 g Arg/ kg of diet thus; the obtained results in relation to blood cells are relatively acceptable.

Serum biochemical parameters

The effects of dietary treatments on serum biochemical parameters of meat-type quails at d 35 are summarized in Table 6. Total protein values were not affected by the dietary (p>0.05). Serum globulin concentration significantly (p<0.05) decreased

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by addition of 1.21 (1.51 g/mL) g Arg/kg of diet compared to those fed basal diet (1.89 g/mL), but didn't differ those fed basal diet supplemented with 0.4 (1.63 g/mL) and 1.84 (1.62 g/mL) g Arg/kg of diet. The urea concentration of serum was significantly decreased by increasing Arg:Lys ratio to 109 (p < 0.05). The uric acid content of serum significantly (p < 0.05) decreased by addition of 1.84 (1.75 mg/dL) g Arg/kg of diet compared to those fed basal diet (2.67 mg/ dL), basal diet supplemented with 1.21 (2.64 mg/ dL). Serum triglyceride concentration significantly decreased by addition of different levels of Arg. Serum total cholesterol was not markedly affected by the dietary treatments (p>0.05). Serum HDL-cholesterol concentration was significantly (p < 0.05) increased by increasing Arg:Lys ratio to 114. Similarly, Yang et al. (2016) reported that laying hen that fed 8.5 mg Arg/kg of diet had significantly lower serum total cholesterol and triglyceride levels compared to the control group. Fouad et al. (2013) reported that the addition of Arg in broiler chickens' diet reduced serum total cholesterol. Al-Daraji et al. (2012) found that the dietary Arg supplementation reduced serum total lipids and triglyceride content of Japanese quails.

 Table 6 – Effect of experimental diets on blood biochemical parameters of broiler quails at d 35.

Blood parameters ¹		Arginin	_			
	100	103	109	114	SEM	p-value
Protein, g/mL	2.90	2.60	2.52	2.69	0.10	0.100
Albumin, g/mL	1.01	0.96	1.00	1.06	0.04	0.570
Globulin, g/mL	1.89ª	1.63 ^{ab}	1.51 ^b	1.62 ^{ab}	0.08	0.060
Urea, mg/dL	6.22ª	4.70 ^{bc}	5.66 ^{ab}	3.76 ^c	0.35	0.002
Uric acid, mg/dL	2.67ª	2.19 ^{ab}	2.64ª	1.75 ^b	0.11	0.001
Triglyceride, g/mL	302ª	182°	199 ^{bc}	218 ^b	8.32	0.001
Total cholesterol, g/mL	163	163	158	150	6.02	0.110
HDL-cholesterol, g/mL	119 ^b	111 ^b	111 ^b	137ª	1.93	0.001

¹HDL-cholesterol = high-density lipoprotein; LDL-cholesterol = low-density lipoprotein. SEM = standard error of mean.

^{a-c}values in the same row not sharing a common superscript differ (p < 0.05).

CONCLUSION

In conclusion, Arg supplementation in the quail diets did not appear to have any positive effects on growth performance; excessive Arg inclusion rate may impair the percentage of PCV and monocytes, eosinophils and lymphocytes values. Some of the serum biochemical parameters including uric acid, total cholesterol and HDL-cholesterol were improved by increasing Arg/Lysine ratio to 114.

DISCLOSURE STATEMENT

We declare that authors don't have any conflict of interest.

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