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The Effect of Calcium and Available Phosphorus Levels on Performance, Egg Quality and Bone Characteristics of Japanese Quails at End of the Egg-Production Phase

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ABSTRACT

Calcium (Ca) and available phosphorus (avP) requirements for egg production are different between the beginning and the end of the egg-production cycle. The objective of this study was to estimate the Ca and avP requirements of Japanese quails at end of the production phase. In total, 256 Japanese quails with 48 weeks of age were randomly distributed in 2 x 2 factorial arrangement consisting of two Ca levels (29 and 38 g/kg) and two avP levels (1.5 and 3.0 g/kg), totaling four treatments with eight replicates of eight hens each. The variables evaluated were feed intake (FI); egg production (EP); marketable egg production (MEP); egg weight (EW); egg mass (EM); feed conversion ratio per egg mass (FCRM); feed conversion ratio per dozen eggs (FCRD); livability (L); yolk weight (YW), albumen weight (AW), eggshell weight (SW); yolk percentage (YP), albumen percentage (AP), eggshell percentage (SP), specific egg weight (SEW); bone calcium percentage (BCa), bone phosphorus percentage (BP), bone ash weight (Bash) and bone ash percentage (PBash). There was no interaction between Ca and avP levels for any of the evaluated parameters, except for SEW. Quail performance was not influenced by the treatments, except for MEP. Bone characteristics were not influenced by the tested dietary Ca and avP levels. However, SW and SP increased and AP decreased as dietary Ca level increased. The dietary supplementation of 38 g Ca/kg and 3.0 g avP/kg may increase marketable egg production and the egg quality of Japanese quails at end of the production cycle.

INTRODUCTION

The knowledge of calcium (Ca) and available phosphorus (avP) requirements are important for older laying quails because they require more minerals than younger birds for bone and egg mineral deposition (Costa *et al*., 2010b). Dietary Ca supplementation is required during the egg production to improve Ca deposition on eggshell, and also it is an important mineral for quail bone development (Garcia *et al*., 2000). Phosphorus is also an important nutrient of quail diets because it is a component of the bone matrix and influences calcium bone deposition (Scott *et al*., 1982). Those nutrients work together during the absorption, metabolism, and excretion (Salter *et al*. 1931). According to Furtado (1991), the best level of phosphorus (P) absorption occurs when dietary Ca level are appropriate according to the adequate Ca: avP ratio. Excessive Ca levels may reduce P absorption by producing an insoluble complex with myo-inositol and trace elements in the intestinal lumen. Therefore, a perfect balance between Ca and avP levels in quail diets are required.

Currently, Ca and avP requirements for Japanese quails can be obtained from published nutritional tables (NRC, 1994; Rostagno *et al*., 2011) as well as from published studies (Costa *et al*., 2007; Vieira *et al.*, 2012; Aguda *et al.*, 2013). However, these requirements were obtained in studies evaluating quails at the beginning of the egg-production phase and during the peak of egg production (Costa *et al.* 2011). Also, there are few studies about Ca and avP requirements for Japanese quails at end of production phase (Costa *et al.,* 2010a; Costa *et al.,* 2010b).

Costa *et al.* (2010a) determined a requirement of 22 g Ca/kg of egg production in Japanese quails immediately after the peak of egg production. However, those authors recommend a higher dietary Ca level (38 g/kg) for maximum eggshell thickness and eggshell percentage. In another study, Costa *et al.* (2010b) estimated a dietary Ca level of 35 g/kg for Japanese quails at end of the egg-production cycle. However, because this result was estimated by a linear regression equation, it is not possible to estimate the maximum Ca recommendation.

The Ca and avP requirement data on the current Brazilian nutritional tables for poultry (Rostagno *et al.*, 2011; Costa & Silva, 2009) were not estimated using hens during the final production phase, and therefore do not allow determining these recommendations for this production phase.

Therefore, the goal of this study was to identify a possible interaction between dietary Ca and P levels and to evaluate the effects of these levels on the performance, egg quality and bone characteristics of Japanese quails at end of the egg-production phase.

MATERIAL AND METHODS

The study was carried out at Poultry Farm of the Animal Science Department of the University of Viçosa, state of Minas Gerais, Brazil. The experimental procedures (Process Number 16/2015) complied with the ethical principles for animal research established by the Brazilian Committee for Animal Experimentation and current Brazilian legislation (Brasil, 2008).

Two hundred and fifty-six Japanese quails (*Coturnix coturnix japonica*), with 48 weeks of age and initial body weight of 187 g (± 0.006), were evaluated during an experimental period of 63 days. Birds were distributed according to a completely randomized design in a 2 x 2 factorial arrangement. Treatments consisted of two Ca levels (29 g/kg and 38 g/kg) and two avP levels (1.5 g/kg and 3.0 g/kg), with eight replicates of eight birds each. Each experimental unit consisted of a cage with eight birds housed at a density of 106 cm^2/bird. Birds were offered feed and water *ad libitum*. A 16-h light:8-h dark lighting program was adopted, according to the recommendations of Albino & Barreto (2003).

Thermometers were distributed inside the poultry house to measure maximum and minimum environmental temperatures. The maximum and minimum temperatures observed were 26.1 ± 0.7 °C and 14.0 ± 1.9 °C, respectively. Air relative humidity was 84.1 ± 2.8% in the morning and 65.7 ± 5.2% in the evening.

A control diet based on corn and soybean meal (193 g/kg crude protein and 2,800 kcal/kg of AMEn) was formulated to meet the quails' requirements according to NRC (1994). Digestible amino acid requirements were supplied using the digestible lysine:amino acid ratios described by Pinto *et al.* (2003), Umigi *et al.* (2008), Pinheiro *et al.* (2008), and Reis *et al.* (2011), because the recommendations are expressed on total amino acids in the NRC table (1994). The ingredients and the calculated nutritional composition of the experimental diets are shown in Table 1.

Table 1 – Ingredients and calculated nutritional composition of experimental diets (g/kg as fed)

Ingredients	Available phosphorus levels (g/kg)			
	1.5		3.0	
	Calcium levels (g/kg)			
	29	38	29	38
Corn	574.3	525.6	574.3	525.6
Soybean meal (45% CP)	321.8	330.7	321.9	330.7
Soybean oil	12.8	29.2	12.8	29.2
Limestone	71.5	94.8	66.3	89.6
Dicalcium phosphate	2.49	2.62	10.6	10.7
Salt	3.20	3.22	3.20	3.22
L- lysine (79%)	2.27	2.11	2.27	2.11
DL-methionine (99%)	4.01	4.08	4.01	4.08
Choline chloride (60%)	1.00	1.00	1.00	1.00
Mineral premix[1]	0.50	0.50	0.50	0.50
Vitamin premix[2]	1.00	1.00	1.00	1.00
Antioxidant[3]	0.10	0.10	0.10	0.10
Inert material[4]	5.00	5.00	2.07	2.07
Calculated composition				
Metabolizable energy (kcal/kg)	2,800	2,800	2,800	2,800
Crude protein (g/kg)	193	193	193	193
Digestible lysine (g/kg)	11.2	11.2	11.2	11.2
Digestible methionine + cystine (g/kg)	9.41	9.41	9.41	9.41
Digestible threonine (g/kg)	6.16	6.16	6.16	6.16
Digestible tryptophan (g/kg)	2.35	2.35	2.35	2.35
Available phosphorus (g/kg)	1.5	1.5	3.0	3.0
Total calcium (g/kg)	29	38	29	38
Ca: avP ratio	19	25	10	13
Total sodium (g/kg)	1.45	1.45	1.45	1.45

[1] Composition/kg of product. Mn - 160g; Fe - 100g; Zn - 100g; Cu - 20g; Co - 2g; I - 2g; vehicle q.s.p.- 1,000 g.;

[2] Composition/kg of product. Vit. A – 12,000,000 IU; Vit D_3 – 3,600,000 IU; Vit. E – 3,500 IU; Vit B_1 – 2,500 mg; Vit B_2 - 8,000 mg; Vit B_6 – 5,000 mg; Pantothenic acid - 12,000 mg; biotin - 200 mg; Vit. K – 3,000 mg; folic acid – 1,500 mg; nicotinic acid – 40,000 mg; Vit. B_{12} – 20,000 mg; selenium - 150 mg; vehicle q.s.p. – 1,000g;

[3] Butylated hydroxy toluene, BHT (99%). [4] Washed sand.

The performance parameters evaluated on this study were feed intake (FI, g/bird/day); egg production (EP, g/kg); marketable egg production (MEP, g/kg), calculated as total number of intact eggs/total number of eggs produced × 100; egg weight (EW, g); egg mass (EM, g/bird/day); feed conversion ratio per egg mass (FCRM, kg of diet/kg of eggs) and per dozen eggs (FCRD, kg of diet/egg dz); livability (L,%), calculated as total number of live birds – total number of dead birds/ total number of live birds × 100.

The following egg quality parameters were evaluated: yolk weight (YW, g); albumen weight (AW, g); eggshell weight (EW, g); yolk percentage (YP, %), albumen percentage (AP, %), eggshell percentage (ESP, %), specific egg weight (SEW, g/cm^3).

The bone characteristics calcium percentage (BCa, %), phosphorus percentage (BP, %), ash weight (Bashw, g), and ash percentage (PBash, %) were evaluated according to methodology described by Sakomura & Rostagno (2007).

Four eggs per experimental unit were randomly collected on days 19, 20, 21, 40, 41, 42, 61, 62, and 63 of the experimental period (63 days) to determine the weight of egg components. Eggs were individually weighed on 0.001-g precision scale. After weighing, eggs were identified and broken. The yolk of each egg was weighed, and the eggshell was washed and dried to determine its weight. Albumen weight was calculated by difference between egg weight, yolk weight, and eggshell weight.

Specific egg weight was determined by immersion in a series of sodium chloride solutions with increasing density, ranging from 1.055 to 1.09 g/cm^3 in 0.005 g/cm^3 intervals, according to Archimedes Principle), as described by Thompson & Hamilton (1982) and Yannakopoulos & Tserveni-Gousi (1986).

At the end of the trial, 96 hens were euthanized to collect the left tibia and left femur to estimate tibia Ca (%), tibia P (%), and tibia ash percentage (%) and weight (g). The analyses were performed according to the methodology described by Silva & Queiroz (2002), at the Animal Nutrition Laboratory of Department of Animal Science, Federal University of Viçosa, Minas Gerais, Brazil.

The main effects of the study were four treatments. The statistical model used was:

$Y_{ijk} = \mu + \alpha_i + \beta j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where:

Y_{ijk} is the observation k of the ith Ca level within the jth avP level;

μ is the overall mean;

α_i is the effect of the ith dietary Ca level;

β_j is the effect of the jth avP level;

$(\alpha\beta)_{ij}$ is the interaction of the ith Ca level i with the jth avP level;

ε_{ijk} is the residual random error.

All data were analyzed using the GLM procedure of SAS statistical package (SAS Institute, 2004) and means were compared by the test of Tukey at 5% probability level. When there was no interaction between Ca and avP levels, the treatments were studied independently.

RESULTS AND DISCUSSION

There was no effect of the different Ca levels ($p > 0.05$) on hen performance (Table 2). However, the interaction between Ca and avP levels influenced ($p < 0.05$) SEW, with the best result observed with the combination of 38 g/kg Ca with 3.0 g/kg avP (Table 4). There were no significant differences among treatments when the high dietary Ca level (38 g/ kg) was fed in combination with the two avP levels evaluated (1.5 g/kg and 3.0 g/kg).

Performance traits were not influenced by the evaluated dietary avP levels, irrespective of Ca levels, except for MEP. The high dietary avP level increased MEP (2.4%) compared to the low dietary avP level. Recent studies have shown avP levels did not influence the egg production of laying quails (Pedroso *et al*., 1999; Costa *et al*., 2011).

Studies have shown FI is not influenced by dietary Ca levels (Pedroso *et al*., 1999, Barreto *et al*., 2007) nor by dietary avP levels (Costa *et al*., 2011). In the present study, dietary Ca and avP levels did not stimulate nor inhibit FI, and neither induced excess or deficiency of these minerals in the diets. Therefore, the evaluated levels and interactions between these minerals may have influenced the experimental results. Phosphorus metabolism is directly related to dietary Ca levels, and it influences bone eggshell Ca deposition in laying Japanese quails and chickens (Costa *et al*., 2007; Almeida *et al*., 2009; Vieira *et al*., 2012).

The evaluated dietary Ca levels influenced ($p < 0.05$) ESW, ESP, and AP. The best ESW and SP results were obtained when hens were fed diets with 38 g Ca/kg. Eggshell weight increased ($p < 0.05$) by 4.5% in birds fed the diets with 38 g Ca/kg compared with those fed 29 g Ca/kg. Also, ESP increased ($p < 0.05$) by 4.1% when the highest Ca level was fed. On the other hand, AP decreased ($p < 0.05$) by 1.4% when birds were fed diets with 38 g/kg Ca compared with the lower dietary Ca level (29 g/kg).

Table 2 – Performance parameters of Japanese quails fed diets with different calcium (Ca) and available phosphorus (avP) levels during 48-57 weeks of age

Factors		Variables [1]							
Ca	avP	FI (g/bird/day)	EP (g/kg)	MEP (g/kg)	EW (g)	EM (g/bird/day)	FCRM (kg/kg)	FCRD (kg/dz)	L (%)
29	1.5	25.6	716.9	949.3	11.68	8.36	3.08	0.43	92.8
	3.0	25.8	770.6	970.3	11.86	9.15	2.86	0.41	94.6
38	1.5	25.7	773.7	955.1	11.59	8.99	2.90	0.40	89.3
	3.0	26.3	804.8	980.4	11.92	9.60	2.75	0.39	89.3
					Mean				
Ca	29	25.7	743.8	959.8	11.8	8.76	2.97	0.42	93.7
	38	26.0	789.3	967.8	11.7	9.29	2.83	0.40	89.3
avP	1.5	25.6	745.3	952.2 b	11.6	8.68	2.99	0.42	91.1
	3.0	26.1	787.7	975.4 a	11.9	9.37	2.81	0.40	91.9
					p value				
	Ca	0.453	0.125	0.404	0.900	0.170	0.148	0.077	0.235
	avP	0.343	0.151	0.020	0.094	0.079	0.061	0.170	0.810
	Ca x avP	0.650	0.697	0.821	0.630	0.813	0.689	0.484	0.810
	SEM²	1.223	8.126	2.625	0.424	1.080	0.268	0.033	10.467

[a, b]Means followed by different letters in the same column are significantly different by Tukey's test at 5% probability level ($p<0.05$).

[1]Feed intake (FI), egg production (EP), marketable egg production (MEP), egg weight (EW), egg mass (EM), feed conversion ratio per egg mass (FCRM), feed conversion ratio per egg dozen (FCRD) and livability (L).

[2]Standard error of the mean.

The influence of dietary Ca levels on ESW, ESP, AP, and SEW supports the dietary requirement of 38 g Ca/kg proposed by Costa *et al.* (2010b) to improve eggshell thickness and eggshell percentage in Japanese quails. According to Scherer (2004), calcium utilization mainly depends on animal species and age. Endogenous calcium is primarily utilized for eggshell formation in laying birds. In addition, daily eggshell calcium deposition represents 10% (2.0 g) of total stored calcium in the body of quails (Miller, 1967). The present study confirms the importance dietary Ca for egg quality, as demonstrated by the ESW, ESP and SEW results. According to Pedroso *et al*. (1999), supplying adequate calcium levels during the egg-production phase may allow maintaining eggshell quality throughout the productive life of Japanese quails.

The effect of dietary Ca levels on AP was different from those on ESW and ESP. Albumen percentage decreased when the diet contained 38 g Ca/kg (Table 3). High Ca levels may increase blood alkalinity by

Table 3 – Egg quality traits of Japanese quails fed diets with different calcium (Ca) and available phosphorus (avP) levels during 48-57 weeks of age.

Factors		Variables[1]						
Ca	avP	YW (g)	AW (g)	ESW (g)	YP (%)	AP (%)	ESP (%)	SEW (g/cm³)
29	1.5	3.47	7.26	0.87	29.79	62.72	7.49	1.073
	3.0	3.53	7.41	0.89	29.85	62.65	7.50	1.072
38	1.5	3.57	7.14	0.90	30.80	61.44	7.75	1.073
	3.0	3.57	7.39	0.93	30.02	62.13	7.85	1.073
					Mean			
Ca	29	3.50	7.34	0.88 b	29.82	62.68 a	7.49 b	1.072
	38	3.57	7.26	0.92 a	30.41	61.79 b	7.80 a	1.073
avP	1.5	3.52	7.20	0.86 b	30.30	62.08	7.62	1.073
	3.0	3.55	7.40	0.91 a	29.93	62.39	7.68	1.072
					p value			
	Ca	0.075	0.535	0.003	0.067	0.010	0.001	0.004
	aP	0.441	0.098	0.046	0.250	0.358	0.517	0.178
	Ca x avP	0.424	0.684	0.498	0.183	0.257	0.620	0.013
	SEM²	0.1058	0.3348	0.0336	0.8736	0.9258	0.2445	0.0009

[a, b]Means followed by different letters in the same column are significantly different by Tukey's test at 5% probability level ($p< 0.05$).

[1]Yolk weight (YW), albumen weight (AW), eggshell weight (ESW), yolk percentage (YP), albumen percentage (AP), eggshell percentage (ESP), specific egg weight (SEW).

[2]Standard error of the mean

interfering with the acid-base balance (Salter *et al.*, 1931). The maintenance of the blood acid-base balance is physiologically and biochemically important, because cell enzyme activity, electrolyte exchange, and the maintenance of body protein structure are affected by small changes in blood pH (Macari *et al.*, 2002). The albumen is composed of 13.5% proteins (FAO, 2010), out of which albumins account for 70% (Ramos, 2008). Serum calcium can be found in the albumen attached to albumin (Vieites *et al.*, 2004). Increasing blood pH may theoretically reduce the blood ionized calcium pool by increasing the concentration of calcium that is bound to protein (Allen & Somjen, 1983), and therefore, reduce the availability of proteins to synthesize the egg albumen.

Egg quality traits were not influenced by dietary avP levels, except for ESW, which increased by 5.8% in birds fed diets with high dietary avP level compared to the low dietary avP level. Eggshell percentage was not affected by dietary avP levels (p>0.05).

Table 4 – Effect of the interaction between dietary calcium (Ca) and available phosphorus (avP) levels on specific egg weight (SEW)

SEW (g/cm³)				
Factors[1]		Ca (g/kg)		
		29	38	Mean
avP (g/kg)	1.5	1.073 Aa	1.073 Aa	1.072
	3.0	1.072 Bb	1.073 Aa	1.072
Mean		1.072	1.072	SEM[2] = 0.0009

[1]Means followed by different uppercase letters in the same column and by different lowercase letters in the same row are significantly different by Tukey's test at 5% probability level (p< 0.05).

[2]Standard error of the mean

There was no statistical effect of treatments on bone characteristics (p> 0.05), which results are shown in Table 5. The effects of dietary Ca and avP levels on the bone matrix are less evident at the end of the egg-production phase than in the beginning probably due reduced intestinal absorption and bone mobilization of these minerals at this age (Costa *et al.*, 2010b). Dietary avP levels did not influence (p> 0.05) bone characteristics.

Some studies have shown that higher eggshell weight and specific egg weight positively influence total intact egg production (Costa *et al.*, 2010a; Costa *et al.*, 2011; Vieira *et al.*, 2012). MEP is the ratio between total number of intact eggs and total egg production; therefore, the higher total intact egg production, the higher the MEP.

The results of the present study indicate that Japanese quails require higher Ca levels than laying chickens at end of production phase. Previous studies have also shown that Japanese quails need higher Ca levels at end of the egg-production phase because quails present faster deterioration of performance and egg quality compared with chickens (Almeida Paz *et al.,* 2009; Pedroso *et al.,* 1999). Costa *et al.* (2010b) reported that the dietary level of 35 g Ca/kg promoted good performance and egg quality in Japanese quails at the end of the egg-production phase.

The dietary avP level of 3.0 g/kg improved marketable egg production and eggshell weight. Additionally, the best egg quality results were obtained with the combination of high dietary Ca level with

Table 5 – Bone characteristics of Japanese quails fed diets with different calcium (Ca) and available phosphorus (avP) levels during 48-57 weeks of age

Factors		Variables[1]			
Ca	avP	BCa (%)	BP (%)	BashW (g)	PBash (%)
29	1.5	38.6	22.0	0.885	62.4
	3.0	37.9	20.9	0.885	61.9
38	1.5	39.1	22.2	0.903	64.0
	3.0	37.6	22.2	0.902	63.7
		Mean			
Ca	29	38.3	21.4	0.885	62.1
	38	38.4	22.2	0.902	63.8
avP	1.5	38.9	22.1	0.894	63.2
	3.0	37.8	21.5	0.894	62.8
		p value			
	Ca	0.957	0.247	0.308	0.138
	avP	0.383	0.363	0.975	0.726
	Ca x avP	0.773	0.358	0.975	0.930
	SEM[2]	2.792	1.335	0.036	2.447

[a, b]Means followed by different letters in the same column are significantly different by Tukey's test at 5% probability level (p< 0.05).

[1]Bone calcium percentage (BCa), bone phosphorus percentage (BP), bone ash weight (BashW) and bone ash percentage (PBash).

[2]Standard error of the means.

high dietary avP. This indicates that when dietary avP levels are increased, Ca levels must be proportionally increased in order to maintain good performance and egg quality.

CONCLUSIONS

The dietary available phosphorus level of 3.0g/kg increases marketable egg production, and high dietary calcium and available phosphorus levels improve the egg quality of Japanese quails at end of the egg-production phase.

The supplementation of 38 g Ca/kg (998 mg Ca/bird/day) and 3.0 g avP/kg (78 mg avP/bird/day) to the diet of Japanese quails at the end of the egg-production phase is recommended.

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