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## Optimal Available Phosphorus Levels in Diets Containing Different Dicalcium Phosphate Particle Sizes for Japanese Quails

### ABSTRACT

This experiment was conducted to investigate the effects available phosphorus levels and dicalcium phosphate particle size on the live performance, and egg parameters and bone parameters of Japanese quails in lay. The experimental diets were based on corn and soybean meal and formulated to contain 2900 kcal metabolizable energy (ME) kg<sup>-1</sup>, 200 g kg<sup>-1</sup> crude protein (CP) and 30.5 g kg<sup>-1</sup> calcium (Ca). Feeds were supplied *ad libitum*. Laying Japanese quails (n=720, 20-wk-old) were distributed in a completely randomized design in a 5 x 2 factorial arrangement, with five levels of available phosphorus (avP; 1.0, 2.0, 3.0, 4.0, 5.0 g kg<sup>-1</sup>) and two dicalcium phosphate particle sizes (fine or coarse), totaling ten treatments with eight replicates of nine birds each. Egg laying rate, feed intake per bird per day, egg weight, egg mass, feed conversion ratio per egg mass and per dozen eggs, eggshell, yolk and albumen relative and absolute weights, as well as bone ash, phosphorus, calcium, and magnesium contents were evaluated. Based on the results, avP recommendations for 20- to 32-wk-old laying Japanese quails varies according with phosphate source particle size and the dietary avP levels of 4.40 g kg<sup>-1</sup> and 3.85 g kg<sup>-1</sup> avP levels when using fine and coarse particle dicalcium phosphate, respectively, yielded the best results.

### INTRODUCTION

Available phosphorus (avP) requirements should be taken into consideration when formulating diets for laying quails, as they have higher requirements than younger birds for bone remodeling and egg deposition (Costa *et al.*, 2010). Phosphorus (P) is a component of the nucleic acids involved in cell growth and differentiation, and it is essential for energy use and transfer (in the form of ATP), fat transport, amino acid and protein synthesis, and also participates in the control of appetite, consequently affecting feed efficiency (Runho *et al.*, 2001). This mineral is the third most expensive nutrient in non-ruminant diets, after energy and protein sources, in particular sulfur amino acids and lysine sources. Dietary P has been extensively researched relative to its economic and physiological importance, but also as a potential environmental polluting agent (Pinheiro *et al.*, 2015).

However, there is limited research on the inclusion of granulated phosphate in the diet of Japanese quails. Griffith & Schexnailder (1970) observed that phosphate sources with large particle sizes increases phosphorus availability to laying hens, as it increases phosphate retention time in the gizzard, allowing its slow release in that acid environment, and consequently improving its digestibility. The solubility of P increases with the degree of grinding of phosphate sources. The eggshell is formed during the night, and part of the ingested P is directed to yolk



deposition and another part is combined with calcium to be deposited in the bone.

The release of calcium from the bone is accompanied by phosphorus, significantly increasing the level of this mineral in the bloodstream. Therefore, special attention should be given to dietary phosphorus levels, because excessive P impairs the release of calcium from the bone and prevents adequate eggshell mineralization. Calcium and P absorption, metabolism, and excretion are closely linked (Salter *et al.*, 1931). According to Furtado (1991), the best level of phosphorus (P) absorption occurs when dietary Ca levels provide for 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, emphasizing the need to obtain adequate balance between Ca and avP levels in quail diets.

Therefore, the objective of this study was to evaluate the effect of dicalcium phosphate particle size and dietary available phosphorus levels on the production performance, egg quality, and bone characteristics of laying Japanese quails.

## MATERIALS AND METHODS

This study was approved by the Animal Welfare Commission of the Federal University of Espirito Santo and conducted in compliance with animal welfare regulations under protocol number 024/2012.

The study was conducted at the Department of Animal Science, Federal University of Espirito Santo, Alegre, SP, from January to April 2013.

The experimental period of 84 days was subdivided into four periods of 21 days. Seven hundred and twenty 20-wk-old Japanese quails were housed in battery cages, and exposed to 16 h light/day, and had *ad libitum* access to feed and water. Birds were randomly distributed according to a completely randomized experimental design in a 5x2 factorial arrangement (five avP levels and two dicalcium phosphate particle sizes), with eight replicates of nine birds each.

Diets were formulated according to Rostagno *et al.* (2011) to contain adequate levels of all nutrients

and energy, except for avP. Dicalcium phosphate (fine or coarse particles) and limestone were added to the basal diet (Table 1) in replacement of inert material (sand) in order to obtain five different avP levels (1.0, 2.0, 3.0, 4.0, 5.0 g kg<sup>-1</sup>) for each particle size (Table 2).

**Table 1** – Ingredients and calculated nutritional composition of the basal diet.

Ingredient	Amount (g kg <sup>-1</sup> )	
Corn	572.30	
Soybean meal 45%	241.34	
Corn gluten 60%	71.84	
Soybean oil	15.52	
Limestone	11.01	
Salt	5.87	
L-Lysine HCL	3.18	
DL-Methionine	1.01	
L-Threonine	0.59	
Choline chloride 60%	0.60	
Vitamin supplement <sup>1</sup>	1.00	
Mineral supplement <sup>2</sup>	0.50	
Zinc bacitracin	0.15	
BHT <sup>3</sup>	0.10	
Inert material (sand)	75.00	
<b>TOTAL</b>	<b>1000.00</b>	
Calculated Composition		
Metabolizable Energy	(MJ kg <sup>-1</sup> )	12.14
Crude protein	(g kg <sup>-1</sup> )	200.00
Digestible lysine	(g kg <sup>-1</sup> )	10.50
Digestible methionine	(g kg <sup>-1</sup> )	4.27
Digestible methionine + cystine	(g kg <sup>-1</sup> )	7.20
Digestible threonine	(g kg <sup>-1</sup> )	7.30
Digestible arginine	(g kg <sup>-1</sup> )	11.14
Digestible tryptophan	(g kg <sup>-1</sup> )	1.90
Calcium	(g kg <sup>-1</sup> )	5.00
Available phosphorus	(g kg <sup>-1</sup> )	1.00
Linoleic acid	(g kg <sup>-1</sup> )	21.33
Sodium	(g kg <sup>-1</sup> )	2.50
Chlorine	(g kg <sup>-1</sup> )	0.07
Potassium	(g kg <sup>-1</sup> )	6.67
Electrolyte balance	(mEqkg <sup>-1</sup> )	166.41

<sup>1</sup> Mineral supplement (per kg of diet): 55000 mg of Zn (min.); 700 mg of I (min.); 10000 mg of Cu (min.); 78000 mg of Mn (min.); 48000 mg of Fe (min.); 200 mg of Co.

<sup>2</sup> Vitamin supplement (per kg of diet): 3.75 mg Folic acid; 60 mg Pantothenic acid; 0.125 mg Biotin; 175 mg Niacin; Vitamin A 40000 UI; Vitamin B1 7.5 mg; Vitamin E 75 UI; Vitamin B1260 mcg; Vitamin B2 25mg; Vitamin B6 14 mg; Vitamin D310000 UI; Vitamin K3 9 mg; 1 mg of Selenium;

<sup>3</sup> Butyl-hydroxy toluene 5 mg.

**Table 2** – Dicalcium phosphate and limestone inclusion levels in substitution of inert material.

avP (g kg <sup>-1</sup> )	Dicalcium phosphate (g kg <sup>-1</sup> )	Limestone (g kg <sup>-1</sup> )	Ca:AvP ratio	Inert material (g kg <sup>-1</sup> )
1.0	0.00	66.41	30:1	8.60
2.0	5.41	62.92	15:1	6.69
3.0	10.81	59.51	10:1	4.69
4.0	16.21	56.07	7:1	2.73
5.0	21.62	52.63	6:1	0.75



Fine (FPP) or coarse (CPP) phosphate particle sizes measured, respectively, geometric mean diameter (GMD) of 148 and 325  $\mu\text{m}$  and geometric standard deviation (GSD) of 0.97 and 2.14. The determination of phosphate particle size was calculated using the equation described by Handerson & Perry (1955).

Birds were allotted to the treatments in order to obtain uniform body weight and egg production among replicates. Average egg weight, egg mass and feed conversion ratio (g of feed per g of egg mass – FCR<sub>em</sub>; and kg of feed per dozen eggs – FCR<sub>dz</sub>) were determined in the eggs collected during the last four days of each 21-d period. Feed intake was determined as the difference between feed provided during the entire experimental period and feed residues at the end of the experimental period.

Bone ash, phosphorus, calcium, and magnesium contents were determined in the right tibiotarsus of

three birds per repetition according to the methodology described by Silva (2009).

The obtained data were submitted to analyses of variance and of regression (polynomial models) using the software SAEG (System for Statistical Analysis and Genetics, 1997) of the Federal University of Viçosa. Independently of the effects of the interaction avP level x phosphate particle size, the results were analyzed in order to determine the effect of phosphorus levels within each studied particle size in order to obtain the avP recommendations as a function of dicalcium phosphate particle size.

## RESULTS AND DISCUSSION

The results showed dietary avP levels has a quadratic effect laying rate (LR) and feed intake (FI), independently of phosphate particle size (Table 3).

**Table 3** – Laying rate (LR), feed intake (FI), and their respective equations of Japanese quails fed diets containing different avP levels from fine (FPP - 148 $\mu\text{m}$ ) or coarse (CPP- 325  $\mu\text{m}$ ) phosphate particle sizes.

AvP (g kg <sup>-1</sup> )	LR (%)		FI (g)	
	FPP	CPP	FPP	CPP
1.0	71.63	71.63	24.55	24.55
2.0	79.35	77.91	24.98	25.29
3.0	78.64	79.20	24.96	25.28
4.0	80.73	78.10	25.86	24.75
5.0	81.70	79.67	25.28	24.61
Significance	$p \leq 0.002$	$p \leq 0.074$	$p \leq 0.020$	$p \leq 0.055$
Effect	Q	Q	Q	Q
CV (%)	6.298		3.557	
Parameter	Equation	R <sup>2</sup>	Max point	Estimated level (%)
LR (FPP)	$LR = 66.61 + 67.40FPP - 76.53FPP^2$	0.88	81.46%	0.44
LR (CPP)	$LR = 66.52 + 66.84CPP - 84.30CPP^2$	0.78	79.77%	0.26
FI (FPP)	$FI = 23.88 + 7.03FPP - 7.83FPP^2$	0.66	25.40g	0.44
FI (CPP)	$FI = 23.89 + 9.33CPP - 16.24CPP^2$	0.77	25.22g	0.28

Q= Quadratic effect; NS= non-significant effect; CV= Coefficient of variation; CPP= coarse particle size; FPP= fine particle size;

The level of 1.0 g kg<sup>-1</sup> of available phosphorus refers to the basal diet with no addition of dicalcium phosphate.

The highest LR response was obtained with 0.44 and 0.26% avP in the diets with FPP and CPP, respectively grain (Table 3). The obtained LR values may be associated with phosphorus role in the absorption of other minerals, mainly calcium and magnesium. In addition, the lower avP recommendation estimated when CPP phosphate was added (Table 3) may be due to the longer retention time of coarse phosphate particles in the gizzard compared with FPP, which readily supply a higher concentration of ionized and solubilized phosphorus (P) in the intestinal lumen. The presence of high P levels in the intestine, as a result of high dietary Ca:P ratio, increases intestinal pH, which results in the formation of P complexes with

calcium, zinc, and manganese, consequently impairing the absorption of these minerals (Oderkirk, 1998). However, Vieira *et al.* (2012) did not observe any significant effect of 0.10 to 0.31% avP levels on the egg production of Japanese quails, neither Amoah *et al.* (2012) with increasing dietary avP levels.

The highest FI was determined in birds fed 0.44% avP from FPP and 0.28% avP from CPP (Table 3), and may be explained by the higher P availability in FPP, releasing P ions in the intestinal lumen, causing greater reaction with other nutrients and forming inorganic phosphates, consequently reducing P absorption (Li *et al.*, 2016; DiMeglio & Imel, 2019). Therefore, birds need to increase their feed intake to supply their



P requirements. At the same time, according to the theory of feed intake regulation (Gonzalez, 2002), high intestinal P levels may saturate P absorption sites, reducing blood P levels because P absorption rate is slower when compared with that of other minerals. Consequently, birds have higher FI to attempt to achieve normal blood P levels. However, Ribeiro *et al.* (2016), evaluating 1.5 or 3.0 g kg<sup>-1</sup> avP levels did not observe any feed intake inhibition or stimulation in Japanese quails.

Egg weight (EW), FCR (per dozen eggs), and egg mass (EM) showed a quadratic response to avP levels, independently of phosphate particle size, while FCR (per g) linearly decreased (Table 4).

The heaviest eggs in birds fed FPP were determined at 0.196% avP level. These results disagree with those reported by Vieira *et al.* (2012), who evaluated different dietary P levels (0.10 to 0.31%) and did not observe any effect egg weight.

Egg mass is the ratio between laying rate and egg weight, and any changes in one of these parameters may have significant effects on egg mass. The estimated avP level when using FPP (0.392%) is very close to that obtained with CPP (0.385%). Therefore, the EM differences obtained are a result to laying rate rather than to egg weight, as laying rate tends to decrease and egg size to increase as birds age, and indicate that laying Japanese quails maintain egg

**Table 4** – Egg weight (EW), egg mass (EM), feed conversion ratio (FCR) per g and per dozen and their respective equations.

avP (g kg <sup>-1</sup> )	EW (g)		EM (g egg/bird/d)		FCR (g/g)		FCR (kg/dz)	
	FPP	CPP	FPP	CPP	FPP	CPP	FPP	CPP
1.0	11.66	11.66	8.35	8.35	2.946	2.946	0.412	0.412
2.0	11.74	11.62	9.31	9.05	2.690	2.768	0.378	0.389
3.0	11.62	11.62	9.13	9.20	2.763	2.733	0.382	0.384
4.0	11.60	11.53	9.37	9.01	2.777	2.753	0.385	0.382
5.0	11.44	11.55	9.34	9.20	2.731	2.708	0.372	0.373
Significance	$p \leq 0.039$	$p > 0.05$	$p \leq 0.02$	$p \leq 0.075$	$p \leq 0.002$	$p \leq 0.011$	$p \leq 0.065$	$p \leq 0.002$
Effect	Q	NS	Q	Q	L	L	Q	L
CV (%)	2.047		6.404		5.723		6.186	
Parameter	Equation		R <sup>2</sup>		Max point		Estimated Level (%)	
EW (FPP)	EW = 11.59 + 1.08FPP – 2.76FPP <sup>2</sup>		0.74		11.70		0.196	
EM (FPP)	EM = 7.71 + 8.73FPP – 11.15FPP <sup>2</sup>		0.90		9.42		0.392	
EM (CPP)	EM = 7.78 + 7.50CPP – 9.73CPP <sup>2</sup>		0.74		9.23		0.385	
FCRem (FPP)	FCCPP = 2.88 – 0.34FPP		0.78		----		> 0.5	
FCRem (CPP)	FCCPP = 0.29 – 0.49CPP		0.59		----		> 0.5	
FCRdz (FPP)	FCKGDZ = 0.43 – 0.25FPP + 0.29FPP <sup>2</sup>		0.81		0.376		0.427	
FCRdz (CPP)	FCKGDZ = 0.41 – 0.09CPP		0.79		----		> 0.5	

CPP= coarse particle size dicalcium phosphate; FPP= fine particle size dicalcium phosphate.

mass for longer than laying rate (Flemming, 2005). However, the EM results disagree with those observed by Amoah *et al.* (2012) and Vieira *et al.* (2012), who evaluated increasing dietary avP levels but did not find any significant effects on egg mass.

Feed conversion ratio per egg mass (g feed/g egg) and per dozen eggs (kg feed/dozen eggs) improved ( $p < 0.05$ ) as dietary avP levels increased, suggesting that P requirements were supplied earlier when quails were fed higher avP levels had more P available. When the diets included CPP, FCR per dozen eggs improved up to 0.427%avP. Garcia *et al.* (2000) observed a linear improvement in FCR per dozen eggs as avP levels increased. Phosphorus metabolism is directly related to the dietary Ca:avP ratio, which influences

feed intake and laying rate in both laying Japanese quails and chickens (Almeida *et al.*, 2009; Vieira *et al.*, 2012).

The effects of dietary avP levels on albumen (ALW and ALRW), yolk (YW and YRW) and eggshell (ESW and ESRW) absolute and relative weights are presented in Table 5. Albumen (FPP) and eggshell (CPP) absolute weights and eggshell relative weight (CPP) present a quadratic response to dietary avP levels, whereas linear responses were obtained for albumen (FPP), yolk (FPP) and eggshell (FPP) relative weights. No influence of avP levels were detected on eggshell (FPP) and albumen (CPP) absolute weights. However, Ribeiro *et al.* (2016) did not find any effect of dietary avP levels on eggshell weight.



**Table 5** – Characteristics of quail eggs.

avP (g kg <sup>-1</sup> )	Absolute weight (g)					
	Albumen		Yolk		Eggshell	
	FPP	CPP	FPP	CPP	FPP	CPP
1.0	6.85	6.85	3.84	3.84	0.96	0.96
2.0	7.12	6.93	3.67	3.74	0.94	0.94
3.0	6.94	6.89	3.74	3.72	0.93	0.98
4.0	6.88	6.90	3.78	3.67	0.94	0.95
5.0	6.83	6.87	3.65	3.74	0.94	0.93
Significance	P ≤ 0.062	p>0.05	p>0.05	p>0.05	p>0.05	P ≤ 0.030
Effect	NS	NS	NS	NS	NS	Q
CV (%)	2.92		4.31		4.17	
avP (g kg <sup>-1</sup> )	Relative weight (%)					
	Albumen		Yolk		Eggshell	
	FPP	CPP	FPP	CPP	FPP	CPP
1.0	58.78	58.78	32.97	32.97	8.24	8.24
2.0	60.68	59.68	31.31	32.20	8.01	8.12
3.0	59.73	59.39	32.24	32.08	8.02	8.51
4.0	59.29	59.82	32.57	31.88	8.12	8.28
5.0	59.79	59.53	31.95	32.41	8.25	8.05
Significance	p≤0.007	p>0.05	p≤0.014	p>0.05	p≤0.066	p≤0.015
Effect	L	NS	L	NS	NS	Q
CV (%)	2.17		3.68		3.75	

CPP= coarse particle size dicalcium phosphate; FPP= fine particle size dicalcium phosphate;

Q= Quadratic effect; NS= no significant effect; CV= Coefficient of variation

The level of 1.0 g kg<sup>-1</sup> of available phosphorus refers to the basal diet with no addition of dicalcium phosphate.

There was no effect ( $p>0.05$ ) of dietary avP levels on the absolute weights of the albumen and yolk, independently of dicalcium phosphate particle size, or of the eggshell of birds fed FPP. Albumen and yolk relative weights of birds fed CPP, and eggshell relative weight of birds fed CPP also did not respond to avP levels ( $p>0.05$ ).

Dietary avP levels had a quadratic effect on ESRW when the diets contained CPP, but no changes were observed when FPP was added to the diets. These results are consistent with those of Costa *et al.* (2010), who observed quadratic eggshell at 0.43, 0.41, 0.42, and 0.27% avP in the diet.

The obtained quadratic response of albumen absolute weight (ALW) to increasing avP level in the diets containing CPP disagrees with Costa *et al.* (2011), who did not find any influence of avP levels on albumen absolute or relative weights.

Dietary avP levels had a linear influence ( $p<0.05$ ) on albumen relative weight (ALRW) and on yolk relative weight (YRW) of quails fed FPP. However, Ceylan *et al.* (2003) and Costa *et al.* (2010) did not find any significant changes in YRW in response to dietary P levels.

The regression equations are shown in Table 6.

**Table 6** – Equations for the estimation of egg quality parameters of Japanese egg quails.

Parameter	Equation	R <sup>2</sup>	Max point	Estimated Level (%)
ESW (CPP)	ESW = 0.92 + 0.36CPP - 0.68CPP <sup>2</sup>	0.90	0.97g	0.26
ARW (FPP)	ARW = 59.47 + 0.62FPP	0.76	-----	> 0.50
YRW (FPP)	YRW = 32.44 - 0.76FPP	0.69	-----	> 0.50
ESRW (CPP)	RESW = 7.88 + 3.44CPP - 6.08CPP <sup>2</sup>	0.58	8.37%	0.28

ESW= eggshell weight; ARW= albumen relative weight; YRW= yolk relative weight; ESRW= eggshell relative weight; CPP= coarse particle size dicalcium phosphate; FPP= fine particle size dicalcium phosphate

The analysis of tibiotarsus parameters (Table 9) shows no influence of dietary avP levels on bone Ca percentage of birds fed CPP, or on P and Mg percentage, independently of dicalcium phosphate particle size. Vieira *et al.* (2012) evaluated four avP

levels (0.10 to 0.31%) combined with three Ca levels (2.0, 2.5, or 3%) in quail diets and did not find any influence on Ca bone content ( $p>0.05$ ). However, those authors reported a linear effect ( $p>0.05$ ) of avP levels in the diets containing 3.0% calcium on



tibial calcium content, whereas Costa *et al.* (2007), evaluating five avP levels (0.15 to 0.55%) in a diet with 3.2% Ca, obtained a quadratic response of P bone percentage.

Bone ash content presented a quadratic response to increasing dietary avP levels (Table 7), with an

**Table 7** – Composition of tibiotarsus of Japanese quails.

avP (g kg <sup>-1</sup> )	Ash (%)		Calcium (%)		Phosphorus(%)		Magnesium (%)	
	FPP	CPP	FPP	CPP	FPP	CPP	FPP	CPP
1.0	52.19	52.19	23.85	23.85	16.38	16.38	1.82	1.84
2.0	52.51	53.71	22.22	21.38	16.65	16.11	2.96	1.98
3.0	54.44	54.14	21.39	21.77	16.28	16.23	1.91	2.10
4.0	56.09	54.38	20.33	21.82	16.23	16.64	2.10	1.88
5.0	53.63	53.99	21.13	22.22	16.35	16.97	1.95	2.16
Significance	P ≤ 0.001	P ≤ 0.055	P ≤ 0.008	p>0.12	p>0.05	p>0.05	p>0.05	p>0.05
Effect	Q	NS	L	NS	NS	NS	NS	NS
CV (%)	2.73		10.72	10.59		24.07		
Parameter	Equation		R <sup>2</sup>	Max point		Estimated Level (%)		
Ash (FPP)	MM = 48.91 + 31.54FPP – 41.80FPP <sup>2</sup>		0.80	54.85%		0.377		
Calcium (FPP)	Ca = 23.99 – 7.34FPP		0.72	-----		> 0.5		

CPP= coarse particle size dicalcium phosphate; FPP= fine particle size dicalcium phosphate.

The level of 1.0 g kg<sup>-1</sup> of available phosphorus refers to the basal diet with no addition of dicalcium phosphate.

On the other hand, Ca bone content linearly decreased as dietary avP levels increased (Table 9) when birds were fed FPP, but not CPP. This may be explained by the fact that, when diets with increasing inclusion levels of fine dicalcium phosphate are fed, P absorption sites are saturated due to the high availability of ionic P (Angel, 2010; Pereira, *et al.*, 2009), compromising Ca to P ratio and leading to the formation of insoluble phosphates, causing the animal to mobilize bone hydroxyapatite to meet its daily requirement (Pereira, *et al.*, 2009). However, Garcia *et al.* (2000) evaluated similar avP levels (0.27 to 0.42%) at four Ca levels (2.5 to 4%) in quail diets, but did not detect any influence of avP levels on Ca bone percentage (p>0.05), possibly because Ca:P ratio was maintained in the evaluated diets.

The results of the present study indicate that the recommended dietary available phosphorus levels for 20- to 32-wk-old laying Japanese quails depends on dicalcium phosphate particle size. When using fine particle size dicalcium phosphate (148 µm), the recommended dietary avP level is 0.44%, which corresponds to an intake of 113 mg avP/bird/day, whereas for coarse particle size dicalcium phosphate (0.325 µm), the avP recommendation is 0.385%, corresponding an intake of 95.3 mg avP/bird/day.

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estimated maximum point at 0.377% avP. This result is not consistent with the findings of Araujo *et al.* (2010), who fed commercial laying hens with diets three avP levels (0.28, 0.38, or 0.48%) from two fine and coarse particle size phosphate and did not detect any significant changes in bone ash content.

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