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Vitamin K supplementation for meat quail in growth of 1 to 14 days old

Suplementação de vitamina K para codornas de corte de 1 a 14 dias de idade

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Abstract

The experiment was carried outto determine the levels of vitamin K for meat quails (*Coturnixcoturnixsp*) from 1 to 14 days of age. Atotal of 2200 birds were used, complete by random experimental design, with 8 treatments, 5 repetitions and 55 meat quails per experimental unit. The levels of vitamin K supplementation were 0; 0.7; 1.0; 1.3; 1.6; 1.9; 2.2; 2.5 mg/kg diets. The performance was measured through weighing weekly from the birds and feed, and at the end of the experiment was carried out collects blood and bones for the assessment of bone quality parameters. The levels of vitamin K supplementation had no influence on performance orbone quality, except thatthe bone density and calcium concentration of the femur and the bone density of the tibia showeda quadratic effect, with estimates of 0.98; 0.92 and 1.18 respectively. The length of the tibia showed a linear increase according to the levels of vitamin K. There was no effect in the concentration of serum calcium, but there was a quadratic effect in the concentration of alkaline phosphatase. The vitamin K supplementation did not affect the performance of the meat quails from 1 to 14 days of age, showing that the amount of vitamin K present in ground corn and soybean meal-based diets is sufficient to meet the needs of the birds' performance.
Key words: Bone density, bone parameters, European quail, nutrition, performance

Resumo

O experimento foi realizado com o objetivo de determinar os níveis de suplementação de vitamina K para codornas de corte (*Coturnix coturnix* sp) de 1 a 14 dias de idade. Foi utilizado um total de 2.200 aves distruibuídas em delineamento inteiramente casualizado, com 8 tratamentos, 5 repetições e 55 animais por unidade experimental. Os níveis de suplementação de vitamina K utilizados foram: 0; 0,7; 1,0; 1,3; 1,6; 1,9; 2,2; 2,5 mg/kg de ração. Para realização do desempenho zootécnico as aves e rações foram pesadas semanalmente, e ao final do experimento foi realizado a coleta de sangue e de ossos para a avaliação de parâmetros relacionados a qualidade óssea. Não foram verificados efeitos (dos níveis de suplementação de vitamina K sobre o desempenho e parâmetros ósseos, exceto para a densidade óssea do fêmur, a concentração de cálcio do fêmur e na densidade óssea da tíbia que apresentaram efeito quadrático, com estimativas de 0,98; 0,92 e 1,18 mg kg^{-1} de ração, respectivamente. O comprimento da tíbia teve aumento linear de acordo com os níveis de suplementação de vitamina K. Não houve efeito na

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concentração de cálcio no soro, porém, houve efeito quadrático (P<0,05) na concentração de fosfatase alcalina. A suplementação de vitamina K não afetou o desempenho de codornas de corte de 1 a 14 dias de idade, mostrando que a quantidade de vitamina K presente em rações a base de milho e farelo de soja é suficiente para atender às necessidades de desempenho das codornas.
Palavras-chave: Codorna européia, densidade óssea, desempenho, nutrição, parâmetros ósseos

Introduction

The growth in the production of meat quail in the country is driven by several factors, among which can be highlighted the low investment required in facilities and the quick financial return, precocity in production, sexual maturity of the birds (35 to 42 days), rapid growth, high productivity and the small spaces required for a large number of animals (PINTO et al., 2002).

The feed represents more than 70% of the total cost of meat quail production, and concern by nutritionists to offer the birds feed with nutritional levels suitable for maximum performance with consequent economic return (FREITAS et al., 2006). Most of the information available regardingthe nutritional requirements of meat quail is still obtained from foreign literature that deals with conditions that are totally different from thosein Brazil (OLIVEIRA et al., 2000).

Among the components of the quail feed, vitamins account for 33% of the total ingredients present, 2.0%of the costs and 0.08% of the weight of poultry feed (McNAUGHTON; MURRAY, 1990), and according to Adams (1982), when the vitamins are excluded from diets, or poorly absorbed, results in vitamin deficiency related diseases and cannot be synthesized by animals.

Vitamin K is essential for the hepatic synthesis of the factors responsible for blood clotting. It is also involved in mineralization and bone formation through the carboxylation of osteocalcin (ZHANG et al., 2003). Vitamin K alsoinfluences the synthesis of protein present in the plasma, kidneys and other tissues (DUTRA DE OLIVEIRA; MARCHINI, 1998).

Since vitamin K cannot be synthesized and the production by intestinal microbiota seems not to be sufficient to perform their functions in the body, Leeson and Summers (2001) report that for broilers there is a need for synthetic vitamin K supplementation (K3). However, the requirement of vitamin K in birds is based on blood coagulation responses, due some coagulation factors be dependent this vitamin, but information on the required amount of vitamin K for bone growth is scarce (PEARSON DEBRA, 2007; FLEMING et al., 1998).

Zhang et al. (2003) studied the effect of vitamin K supplementation onbroilers and concluded that there is a positive effect on bone quality and feed efficiency.

Given the above, it is believed that vitamin K supplementation is very important in preventing bone problems, and thus future problems in production creation. So this study aimed to evaluate the effect of supplementation of different levels of vitamin K on performance and bone parameters of meat quails of 1 to 14 days old.

Materials and Methods

The experiment was conducted at the Quail Production Area of Iguatemi Experimental Farm at the State University of Maringá – UEM, according to the rules proposed by the ethical conduct committee on the use of animals in experiments bythis university (Protocol 036/2014).

The experimental design was completely randomized with 8 treatments with 5 repetitions and 55 quails per experimental unit. The treatments consisted of the supplementation of eight levels of vitamin K (0, 0.7, 1.0, 1.3, 1.6, 1.9, 2.2, 2.5 mg / kg diet).

The experimental diets were formulated based on ground corn and soybean meal to meet the nutritional requirements, following recommendations by Scherer et al. (2011) for metabolizable energy requirement; Furlan et al. (2011) to meet the requirement of digestible lysine; and Silva et al. (2009) to meet the requirements of calcium and available phosphorus in the diet.The isonutritive diets differed only with respect to the levels of vitamin K, using ground rice huskas the vehicle (Table 1). The source of vitamin K used was Kavist® Plus from Dirox, with menadione (synthetic compound) being the compound used with a composition of 500mg of vitamin K/g. Throughout the experimental period, diet and water were provided for the quail *ad libitum*. Natural light was provided more than artificial light, totaling 24 hours of light during the experimental period.

Table 1. Percentage composition and calculated the experimental diets for quails 1 to 14 days old.

Diet	(g.kg^{-1})
Soybean meal (45%)	53.05
Corn	37.74
Soybean oil	5.6
Bicalcium phosphate	1.46
Salt	0.46
DL-met (99%)	0.45
Calcareous	0.41
Vitamin and mineral mixture[1]	0.4
L- lys HCL	0.21
L- thr	0.11
Vitami K mixture[2]	0.1
Antioxidant[3]	0.01
Calculated values	
Metabolizable energy (kcal.kg^{-1})	2,997
Availabre phosphorus (g.kg^{-1})	4.1
Calcium(g.kg^{-1})	6.5
Crude protein (g.kg^{-1})	275
Digestible lys (g.kg^{-1})	16
Met + digestible cys (g.kg^{-1})	11.5
Digestible thr (g.kg^{-1})	10.4
Digestible tri (g.kg^{-1})	3.3
Chlorine (g.kg^{-1})	3.2

[1]Vitamin and minetal mixture exempt from vitamin K (guarantee levels per kg of product): Vit. A – 2250,000; Vit. D3- 500 UI; Vit. E – 2,000 UI; Vit. B1 – 312 mg; Vit. B2 – 1,000 mg; Vit. B6 – 495 mg; Vit. B12 – 3,333 mcg; D-caldium pantothenate – 3,166 mg; Niacin – 6,533 mg; Folic acid – 133 mg; Biotin – 16.0 mg; Choline – 100 mg; BHT – 1.890 mg; Zinc oxid – 15.0 g; Ferrous – 13.0 g; Manganese – 17.0 g; Copper – 3,000 mg; Iodate – 279 mg; Cobaltous – 56 mg; Selenite – 81.0 mg; Carrier q.s.p. 1,000 g.
[2]Diluitions of vitamin K3 form the desired levels (0.7mg.kg^{-1}; 1,0mg.kg^{-1}; 1.3mg.kg^{-1}; 1.6mg, kg^{-1}; 1.9mg. kg^{-1}; 2.2mg. kg^{-1}; 2.5mg. kg^{-1}).
[3] BHT(Butylated hydroxytoluene).

To evaluate the growth performance, the quails were weighed weekly and simultaneously were carried out the weigh the experimental diets provided to determine the feed intake (g/bird), body weight (g), body weight gain (g), feed conversion (g/g) and accumulated body biomass (%) obtained according to the weight gain in relation to the initial weight at the beginning of the experiment.

Two birds per experimental unit were used for blood tests at the end of the experiment, these being subjected to fasting for 6 hours prior to the blood tests. Blood collection was performed viathe ulnar vein.The samples were placed in test tubes and centrifuged immediately at 3000 rpm for 15 minutes. Serum was separated and placed in identified Eppendorf tubes and stored at –20 ° C until time of analysis. The dosage of the enzyme alkaline phosphatase and the concentration of calcium in the blood were carried out in a spectrophotometer (model BIOPLUS 2000) using commercial kits (Analyzes Gold Diagnostic Ltd.).

Later, these same birds were slaughtered for the analyses of bone parameters made in the femur and right tibia (Seedor index, densitometry, resistance and ash and calcium content).

After collection, the bones were identified and frozen (-18°C) until the beginning of the analysis of bone parameters. To start the analysis, the bone were then thawed, and the surrounding tissues (adhered muscle tissue) were removed using scissors and tweezers.

To determine the Seedor index (SEEDOR et al., 1996), the femur and tibia were weighed using a precision scale and the length was measured with a digital caliper. This index serves as an indicator of bone strength, and this value was found by dividing the weight of the bone by its length.These bones were immersed in petroleum ether for 24 hours to be degreased and then dried in a forced-air oven at 55 ° C for 72 hours beforecarrying out other bone analyses.

The optical density of radiographic determination was performed using the X-ray dental appliance DabiAtlante ®, model Spectro 70X electronic (DabiAtlante, RibeirãoPreto, Brasil), and bone parts placed under film (mark Kodak Intraoral E-Speed Film, size 2, periapical type), all in the same position. After obtaining the radiographs, the processing of the X-ray film was performed by means of an automatic processor (Revel Industry and Equipment Trade Ltd.), with a work time of 150 seconds and operating with Kodak RP X-Omat solutions.

Scans of the radiographs were made in the Image Tool® program (version 3.0, University of Texas Health Science Center at San Antonio, UTHSCSA, USA, ftp://maxrad6.uthscsa.edu/) and recorded in files with a progressive JPG extension. Subsequently, the radiographs were read to determine the density of the bone specimens using "Adobe Photoshop CS6" using a "histogram" tool. The determination of bone density was measured by selecting the average of three selected central points of the bone with a fixed size of 10px x 10px.

For radiographic reference an aluminum scale of 10 degrees with 1 mm of thickness between degreeswas used. The data obtained in gray values was converted to relative values, based on the aluminum scale thickness, while all data were compared to the third degree on this scale.

The bone resistance was analyzed in a press designed to test the compressive unconfined strength of cohesive soil samples, and the values were expressed in kilogram force (kgf).The bone pieces were placed in the anteroposterior position in support of the epiphyseal region, getting the same without support in the central region. The force was applied tothe central region of all the bones; the descent probe speed that applied the force was5 mm / second and the used load was 500 N (Newton), the applied force being measured at the moment before rupture bone.

Finally, these bones were weighed on an analytical balance (0.0001g), dried in ovens at 105 °

C for 24 hours, weighed again to determine the dry matter content, calcined in a muffle at 500 ° C for 5 hours, weighed again to determine the level of ash and then the methodology described by Silva and Queiroz (2002) for determining calcium levels.

The variables were evaluated using the statistical program SAEG (UFV, 2000), performing ananalysis of variance and polynomial regression (P <0.05).

Results and Discussion

There was no effect (P> 0.05) of supplementation levels of vitamin K on feed intake (FI), body weight (BW), weight gain (WG), accumulated body biomass (ABB) or feed conversion (FC) during the period 1 to 14 days (Table 2).

Table 2. Mean values of meat quails performance 1 to 14 days of age depending on the supplementation levels vitamin K.

Vit. K (mg/Kg)	FI (g/ave)	BW (g)	WG (g)	ABB (%)	FC (g/g)
0.00	143.17	84.66	76.22	903.98	1.88
0.70	141.37	83.68	75.24	891.03	1.88
1.00	142.70	84.51	75.95	886.43	1.88
1.30	142.16	83.33	74.91	889.90	1.90
1.60	142.04	86.15	77.66	914.45	1.83
1.90	141.98	84.79	76.34	903.48	1.86
2.20	143.50	83.92	75.62	911.24	1.90
2.50	143.97	85.13	76.58	895.34	1.88
P value	0.6233	0.5372	0.5081	0.3868	0.9500
CV%	3.409	2.667	2.824	2.542	2.962

Coefficient of variation (CV); Feed intake (FI); Body weight (BW); Weight gain (WG); Accumulated body biomass (ABB); Feed conversion (FC).

This can be explained, probably, in function the availability this vitamin in the ingredients provided in the experimental diets. The diet without supplementation (0%) may have been sufficient to provide the required amount of vitamin K, since it didnot differ from other levels.Another explanation for the lack of significance may be attributed to the use of vitamin K2, supplied by microflora in the large intestine, where the bird can absorb this vitamin. McDowell (1989) reports that vitamins can be synthesized by bacteria in the intestinal tract in sufficient quantities, and this may be the case forvitamin K for meat quails from 1 to 14 days of age.

Studies on the effects of vitamin K supplementation on the performance of meat quail, as well as broilers, are scarce. Currently, the recommended levels of at vitamin K for broilers in the initial growth phase is 0.50 mg (NRC, 1994), 1.88 mg (1 to 7 days old) and 1,65mg (8 to 21 days of age) (ROSTAGNO et al., 2011). For European quails the NRC (1994) and Silva and Costa (2009) recommend 1.5 and 0.55 mg of vitamin K, respectively.

Zhang et al. (2003) did not find significant differences in the performance of broilers when working with six vitamin K supplementation levels (control diet, 0.5, 2.0, 8.0, 32.0, and 128.0 mg kg^{-1}). Likewise, Jin et al. (2001), supplementing the diet of 1 to 14-day-old turkeys, detected no significant effect on weight gain, feed intake and feed conversion.

Askim et al. (2012) studied the intake of menaquinone (K2) in the diet of22-day-old broilers and observed that groups with a higher intake of this source of vitamin K showed lower growth, concluding that there is no need for supplementation provided there is a natural source.

The calcium concentration in serum (CCS) was not affected (P> 0.05) by the vitamin K supplementation levels, however, the concentration of alkaline phosphatase (CAP) had a quadratic effect with increasing the level of supplementation, with estimate of 0.17 mg kg $^{-1}$of vitamin K in the diet (Table 3). Parthemore et al. (1993) affirm that because of dissemination into the blood, alkaline phosphatase is a good indicator of bone formation rate. Osteoblasts secrete large amounts of the enzyme alkaline phosphatase in the blood, indicating active inorganic phosphate deposition in the bone matrix, and it is therefore a good indicator of bone formation (SWENSON; REEC, 1993). Minafra et al. (2008) found no significant differences in vitamin K supplementation in calcium and alkaline phosphatase levels in broilers at 21 days of age.

Table 3. Mean values blood parameters of meat quails at 14 days of age depending on the supplementation levels vitamin K.

Vit. K (mg/Kg)	CCS (mg/dL)	CAP (U/L)	
0.00	6.45	2058	
0.70	6.36	2164	
1.00	7.06	2222	
1.30	6.82	1944	
1.60	7.2	1835	
1.90	5.35	1674	
2.20	5.51	1623	
2.50	6.96	1574	
P value	0.2210	0.0140	
CV%	11.967	9.952	
Regression Equation	R^2	Effect	Estimate
CAP= 2127,09 + 0,43077x – 0,0124957x²	0.82	quadratic	0.17

Coefficient of variation (CV); Calcium concentration in serum (CCS); Concentration of alkaline phosphatase (CAP).

The analyzed bones showed a quadratic effect (P <0.05) in the bone density of the femur (BDF), in the femoral calcium concentration (FCC) and in the bone density of the tibia (BDT), with estimates of 0.98; 0.92 and 1.18 mg kg-1 of vitamin K in the diet, respectively. The length of the tibia (LENGT) had a linear increase (P <0.05) according to the vitamin K supplementation levels. The vitamin K supplementation did not affect the weight, diameter, Seedor index, bone resistance or ash concentration in the femur and tibia of these birds, nor the length of the femur orthe calcium concentration of the tibia (Table 4).

Zhang et al. (2003), working with vitamin K supplementation levels, detected that in broiler chickens aged three weeks, bone quality is improved, and the tibia ash concentration increased linearly. On the other hand, they did not observe a significant effect on the weight and length of the tibia, unlike the evaluation in this study where there was a linear increase in the tibia of the quails supplemented with vitamin K. These authors also evaluated mineral density, getting a quadratic effect, agreeing with the result of this research.

Table 4. Mean values bone parameters of quails at 14 days of age depending on the supplementation levels vitamin K.

Vit. K (mg/Kg)	WEIGHT (g)	DIAM (mm)	LENG (mm)	SI (mg/mm)	BD (mm Eq/Al)	BR (kgf)	AC (%MS)	CC (%MS)
				Femur				
0.00	0.46	2.18	31.15	14.82	1.66	23.28	37.37	8.85
0.70	0.47	2.05	30.82	15.31	1.76	18.63	39.57	10.29
1.00	0.50	2.11	31.11	16.13	1.75	18.09	40.02	9.54
1.30	0.48	2.12	31.53	15.15	1.77	20.25	37.78	9.21
1.60	0.51	2.18	31.22	16.36	1.69	24.25	36.89	9.08
1.90	0.44	2.16	30.78	14.34	1.66	24.32	36.86	8.93
2.20	0.45	2.14	31.94	14.13	1.63	22.35	39.53	8.66
2.50	0.47	2.24	31.53	15.08	1.56	21.95	37.99	8.48
P value	0.7260	0.3898	0.3043	0.5822	0.0001	0.2263	0.9135	0.0052
CV%	13.895	3.418	3.418	13.337	4.608	15.911	9.812	7.417
				Tibia				
0.00	0.61	2.02	40.11	15.23	1.80	21.47	41.89	11.47
0.70	0.59	2.01	39.26	15.06	1.91	20.09	42.33	12.06
1.00	0.67	1.99	40.06	16.58	1.95	20.05	41.92	11.57
1.30	0.60	2.05	40.07	15.04	1.97	20.25	41.47	11.35
1.60	0.65	2.00	39.85	16.18	1.88	23.10	42.42	11.51
1.90	0.60	2.01	39.92	14.88	1.87	24.62	43.07	11.81
2.20	0.64	2.00	40.82	15.66	1.81	22.56	41.89	12.64
2.50	0.62	2.01	41.07	14.81	1.81	21.88	42.53	11.75
P value	0.7974	0.9646	0.0501	0.8034	0.0023	0.1536	0.6401	0.3538
CV%	15.076	6.393	3.243	13.24	5.606	15.72	5.904	8.738

Regression Equation	R^2	Effect	Estimate
BD Femur= $1,67404 + 0,00166674x – 0,00000854165x^2$	0.95	quadratic	0.98
CC Fêmur= $9,099778 + 0,00918234x – 0,0000497838x^2$	0.64	quadratic	0.92
Leng Tíbia = $39,5315 + 0,00436983x$	0.41	linear	
BD Tìbia = $1,81155 + 0,00202865x – 0,00000860518x^2$	0.82	quadratic	1.18

Coefficient of variation (CV); Diameter (DIAM); Length (LENG); Seedor index (SI); bone density (BD); , bone resistance (BR); ash concentration (AC); calcium concentration (CC).

By working with four vitamin K supplementation levels for turkeys, Jin et al. (2001) found no significant differences in the ashes of the tibia at 7 days of age. However, when assessing only the control group and the group with the highest vitamin K supplementation (2.0 mg), they observed higher concentrations of ash in the supplemented group.

In contrast, Rodrigues et al. (1996) did not detect significant differences in vitamin K levels in the concentrations of ash in the tibia and femur of broilers at 14 days of age, nor did they obtain significant differences in the calcium concentration and length of these bones.

Askim et al. (2012) found no significant differences in bone strength of broilers supplemented with menaquinone at 22 days of age.

Vitamin K is widely studied because the carboxylation of this vitamin is involved in bone metabolism, after all this vitamin is needed for carboxylation of glutamic acid, component of bone proteins, such as osteocalcin, and these carboxylated proteins have a higher affinity for calcium and are important in the incorporation thereof into the bone (BOOTH; MAYER, 2000; CARBALLO et al., 1999).

Shearer (1995) reports that vitamin K is important in early skeletal development and maturation in the bone. The results of this work show that vitamin K supplementation affects the bone, and that supplementation with 1.18 mg kg^{-1} of vitamin appears to increase bone mineralization.

Conclusion

Vitamin K supplementation did not affect the performance meat quails from 1 to 14 days of age, showing that the amount of vitamin K present in ground corn and soybean meal-based diets is sufficient to meet the needs of the performance of meat quails.

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