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Calcium requirement and vitamin D supplementation in meat-type quail at second stage of growth

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ABSTRACT - An experiment was conducted to determine the required levels of supplementary vitamin D and calcium in meat-type quail (*Coturnix coturnix* sp) between 15 and 35 days old. The experiment was a 4 × 4 factorial design with four levels of calcium: 0.42, 0.58, 0.74, and 0.90% and four levels of vitamin D: 1,000; 2,000; 3,000; and 4,000 IU. Body weight and body weight gain increased linearly and feed conversion showed some linear improvement due to increased levels of Ca and vitamin D. The increased vitamin D levels resulted in a linear increase in feed intake. Calcium and vitamin D requirements in meat-type quail between 15 and 35 days of age is greater or equal to 0.90% Ca and greater or equal to 4,000 IU of vitamin D, probably because the experiment was conducted during the pre-laying phase.

Key Words: bone resistance, nutritional requirement, quail nutrition

Introduction

Investments in quail production in Brazil have increased due to the fact that some characteristics of these birds have become a profitable alternative in the Brazilian market. The increase in quail production has been highlighted over the last years. Its evolution has been constant and increasing numbers of poultry companies have expressed an interest in improving their product quality at a low cost and in meeting the needs of the consumer (Bertechini, 2009).

The market is expanding and, as a result, companies related to quail production need scientific information and data pertaining to nutrition, genetics, management, and ambience of meat-type quail to enhance their production.

An accurate determination of nutritional requirements is of great importance to all bird species, as diet is perhaps the main environmental factor that determines bird growth and allows them to reach their maximum genetic potential (Albino and Barreto, 2003).

Calcium deposition in the skeleton is greater during the grower phase. Thus, calcium content in the body of chicks increases quickly in the starter stage, so that at the end of the first month of age the chicks have 80% of the total calcium of an adult bird (Edwards Jr, 2000).

As the majority of vitamins are not synthetized in large amounts enough to meet the body physiologic demands, these nutrients must be obtained from the diet. According to Combs Jr (2008), the vitamins: are essential, usually in minute amounts, for normal physiological function (i.e., maintenance, growth, development, and/or production); cause, by its absence or underutilization, a specific deficiency syndrome; and are not synthesized by the host in amounts adequate to meet normal physiological needs.

The most physiologically important function of vitamin D is in the homeostasis of Ca^{2+} and phosphate, which is affected by a multihormonal system involving the controlled production of $1,25(OH)_2D_3$, which functions in concert with parathyroid hormone and calcitonin. Regulation of this system occurs at the points of intestinal absorption, bone mineral accretion and mobilization, and renal excretion (Combs Jr, 2008).

Therefore, an experiment was conducted to estimate calcium requirements and vitamin D supplementation levels needed to attain the maximum productive performance and bone development in meat-type quail between 15 and 35 days old.

Material and Methods

The work was conducted in accordance with ethical standards and approved by the Ethics and Biosafety

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Committee of the State University of Maringá (case no. 091/2012).

We kept 1,920 meat-type quail (*Coturnix coturnix* sp) between 15 and 35 days old in a conventional hangar divided into 48 boxes.

The experimental design was completely randomized in a 4 × 4 factorial scheme (four calcium levels: 0.42, 0.58, 0.74, and 0.90% Ca and four vitamin D levels: 1,000; 2,000; 3,000; and 4,000 IU) with a total of 16 treatments, three repetitions, and 40 quail per experimental unit.

An adequate number of repetitions can increase the experimental precision and therefore improve the capacity of a statistical test to detect small differences among estimates of a mean treatment (Velini et al., 2006). On a practical note, Pimentel-Gomes (2000) suggested that an experiment must be designed to provide a minimum of ten degrees of freedom per residue.

Data regarding the temperature was collected early in the morning and in the late afternoon during all experimental periods by using a maximum and minimum thermometer. The average temperature of the experimental period was 22 °C.

The experimental diets were formulated based on ground corn and soybean meal, while the different calcium contents in the diet were obtained by varying the amount of inert limestone, calcium phosphate, and kaolin (Table 1).

To supply the required phosphorus in this study, we adopted the recommendation of Silva et al. (2009) of 4.1 g/kg available P.

The experimental diets were formulated to meet the requirements proposed by Rostagno et al. (2011) for broilers in the starter phase from 1 to 21 days of age, with the exception of calcium and vitamin D. The chemical composition and energy values of feeds were obtained from Rostagno et al. (2011).

Quails were weighed weekly to evaluate performance, while the experimental diets were weighed to determine feed intake (FI) (g/bird), body weight (BW) (g), body weight gain (WG) (g), and feed conversion (FC) (g feed/g gain).

Two quail (one male and one female) per experimental unit were used to determine carcass yield at 35 days of age.

Two quail per experimental unit were chosen for evaluation of bone parameters.

The femur and tibia were weighed using a precision scale and the length was measured with a digital caliper ruler to determine the Seedor index (Seedor et al., 1996).

$$\text{Seedor index} = \text{bone weight (mg)}/\text{length (mm)}$$

The bones were immersed in petroleum ether for 24 h to be defatted. Afterwards, they were dried in a forced-air oven at 55 ºC for 72 h.

The radiographic optical density was determined at a dental clinic.

First, the bones were placed in the same position under a film (Kodak Intraoral E-Speed Film, size 2, periapical type) and were radiographed using the X-ray dental appliance DabiAtlante®, model Spectro 70X electronic (DabiAtlante, Ribeirão Preto, São Paulo, Brazil). The appliance operated at 70 kVp, 8 mA, with a 0.2-s exposure time, determined by a previous pilot test, containing a stepwedge, focusing the X-ray beam perpendicularly to the film at a focus-film distance of 10 cm.

After that, the radiographic films were processed using an automatic processing machine (Revel Industry and Equipment Trade Ltd.) with a work time of 150 s and operating with Kodak RP X-Omat solutions.

In a second step, the radiographic films were scanned into the Image Tool® program (version 3.0, University of Texas Health Science Center at San Antonio, UTHSCSA, EUA, ftp://maxrad6.uthscsa.edu/) and recorded in files with a progressive JPG extension.

Table 1 - Composition of the basal diet offered to quail during the second growth stage

Diet	Quantity (g/kg)
Corn	489.5
Soybean meal (45%)	418.3
Soybean oil	45.2
Monocalcium phosphate	14.4
Vitamin D3[1]	1.0
Limestone + inert substance[2]	14.0
Vitamin and mineral premix[3]	4.0
Salt	4.0
DL-met	4.3
L-lysine HCL	3.6
L-threonine	1.6
Antioxidant[4]	0.1
Calculated values	
Metabolizable energy (mJ/kg)	12.711
Crude protein (g/kg)	235
Available P (g/kg)	4.1
Digestible lysine (g/kg)	14.5
Digestible methionine + cystine (g/kg)	10.4
Digestible threonine (g/kg)	9.4
Digestible tryptophan (g/kg)	2.7
Cl (g/kg)	2.8
Na (g/kg)	1.8
K (g/kg)	9.0

[1] Vitamin D3 (500,000 IU/g) was diluted with rice straw to achieve desired levels (1,000; 2,000; 3,000; or 4,000 IU/kg diet).
[2] Used 14.0 g inert substance (Kaolin, Nucleopar, Mandaguari, Brazil) + 0 g limestone, 9.8 g inert substance + 4.2 g limestone, 5.6 g inert substance + 8.4 g limestone, and 1.4 g inert substance + 12.6 g limestone to achieve dietary Ca concentrations of 0.42, 0.58, 0.74, and 0.90% Ca, respectively.
[3] Vitamin/mineral premix (guaranteed levels per kg of product): retinol acetate, 700,000 IU; dl-α-tocopheryl acetate, 6,250 IU; thiamine hydrochloride, 350 mg; riboflavin, 1,250 mg; pyridoxine hydrochloride, 600 mg; cyanocobalamin, 3,000 mcg; menadione nicotinamide bisulphite, 600 mg; D-calcium pantothenate, 3,000 mg; niacin acid, 8,760 mg; folic acid, 175 mg; biotin, 17.5 mg; choline chloride, 75 mg; butylated hydroxytoluene, 1,000 mg; zinc oxide, 12.0 g; ferrous sulfate, 12.5 g; manganese sulfate, 14.5 g; copper sulfate, 1,000 mg; potassium iodate, 250 mg; cobalt sulfate heptahydrate, 50 mg; sodium selenite, 62.5 mg; and excipient q.s., 1,000 g.
[4] BHT - butylated hydroxytoluene.

Afterwards, Adobe Photoshop CS6 software was utilized to read the radiographic films and to determine the bone density using the Histogram tool, which analyzes the radiographic density of a selected area that is distributed in a color scale, close to gray, and has 256 shades. The value 0 represents the color black while the value 256 represents the color white. Three central points with a fixed size (10 px × 10 px) were selected from the bone and a mean was obtained. The central area was chosen because it is the same area in which the bone received the force required to break in the resistance assay.

An aluminum scale of 10 degrees, with 1-mm thickness between degrees, was utilized as a radiographic reference. The data obtained in gray values were converted to relative based on the aluminum thickness scale, 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 bones were placed in such a way as to support the epiphyseal region, whereas the central region did not have support. The anteroposterior position was chosen to prevent movement of the bone during the break time. The force was applied in the central region at the same point for all bones and the descent probe speed that applied the force was consistent (5 mm/s) for all bones. We used a load of 500 N (Newton) for all samples.

After the bone resistance assay, the left femurs were ground and dried in a forced-air oven. The samples were then weighed using an analytical scale (0.0001 g) and dried in an oven at 105 ºC for 12 h to determine the calcium and phosphorus contents in the bone, using the methodology described by Silva and Queiroz (2002).

The data were analyzed using the Statistical Analysis and Genetic System Program – SAEG (version 9.1) from the Federal University of Viçosa.

First, calcium and vitamin D requirements were estimated by a quadratic model or a discontinuous linear response plateau according to the adjustment data for each variable.

Results were obtained from the response surface analysis, which is a collection of mathematical and statistical techniques used to analyze problems pertaining to the influence of independent variables on response-dependent variables, with the ultimate goal of optimizing responses (Montgomery, 2009).

Results

No interaction was observed (P>0.05) between FI, as a function of calcium, and vitamin D levels in quail between 15 and 35 days of age. Only vitamin D levels caused a linear increase in FI (Table 2).

We observed (P<0.05) a linear increase in calcium and vitamin D requirements regarding the variables BW, weight gain (WG), and FC.

Calcium and vitamin D levels did not affect (P>0.05) phosphorus content in the bone, tibial bone resistance, or bone densitometry (Table 3).

Calcium levels in the diet did not affect (P>0.05) calcium and ash contents in the bones, while vitamin D had a quadratic effect (P<0.05) on the same parameters, for estimated levels of 2,712 and 2,700 IU of vitamin D, respectively.

The femoral Seedor index increased linearly (P<0.05) due to calcium levels, while a quadratic effect (P<0.05) was observed as a result of vitamin D levels, with an estimated level of 2,756 IU of vitamin D. The tibial Seedor index and femoral bone resistance increased linearly (P<0.05) with increased levels of dietary calcium

The blood calcium levels (BLC) increased linearly (P<0.05) with the increase in dietary calcium levels and a quadratic effect (P<0.05) was observed with the increase in vitamin D (VD) according to the following equation: $BLC = 5.86509 - 6.77668 \times Ca + 0.00457851 \times VD - 0.000000976975 \times VD^2$ ($R^2 = 0.91$), with an estimated level of 2,343 IU of vitamin D (Table 3).

Calcium and vitamin D levels did not have significant effects (P>0.05) on carcass and cut yields. However, the same pattern was observed with WG and BW (Table 2) such that calcium and vitamin D levels led to a linear increase (P<0.05) in carcass weight, breast weight, and leg weight.

Discussion

According to Silva and Costa (2009), the calcium supplementation requirement for Japanese quail from 22 to 42 days of age is 0.50%, while for European quail the requirement is 0.70%, in diets containing 3,050 kcal of metabolizable energy (ME)/kg and 22% crude protein (CP). However, Rostagno et al. (2011) recommended 0.90% calcium for Japanese quail in the starter and grower phases, while there was no recommendation reported for vitamin D.

The INRA (1999) tables stated that the calcium requirement is 0.85, 0.90, and 0.95% Ca in diets containing 2,800; 3,000; and 3,200 kcal of ME/kg and 23.0, 24.6, and 26.3% CP, respectively. Moreover, the NRC (1994) reported a requirement of 0.80% Ca in diets containing 2,900 kcal of ME/kg and 24% CP. The experimental diet was formulated to contain 3,036 kcal of ME/kg and 23.5% CP.

Table 2 - Effect of Ca and vitamin D on growth performance and carcass yield of quails from 15 to 35 d of age

Calcium (%)	0.42				0.58				0.74				0.90				CV (%)
Vitamin D (IU)	1,000	2,000	3,000	4,000	1,000	2,000	3,000	4,000	1,000	2,000	3,000	4,000	1,000	2,000	3,000	4,000	
FI (g/quail)	454.7	453.8	490.2	507.2	498.1	468.6	477.0	476.6	454.4	482.2	483.7	493.9	474.5	470.1	481.6	480.8	3.49
BW (g)	214.9	214.4	221.5	220.6	220.5	222.9	218.0	221.1	220.3	218.5	223.6	222.5	224.0	224.7	229.7	227.0	1.79
WG (g)	131.8	127.1	136.6	135.4	136.3	139.6	134.0	137.1	136.1	134.0	139.2	138.0	139.9	141.2	144.5	142.5	2.81
FC (g/g)	3.37	3.57	3.59	3.59	3.41	3.36	3.56	3.48	3.34	3.60	3.47	3.58	3.39	3.33	3.34	3.38	3.48
CW (g)	134.3	127.7	132.8	140.5	138.8	137.3	142.9	134.4	132.8	132.8	144.9	141.4	142.6	140.9	143.1	144.2	3.63
BrW (g)	56.29	55.81	57.53	60.90	59.91	60.09	61.53	60.06	57.69	58.21	61.63	61.84	63.15	60.48	60.59	64.05	5.63
LW (g)	33.78	31.33	33.41	35.21	33.68	33.07	34.13	33.27	32.17	32.62	34.97	34.36	34.15	34.64	35.22	35.04	4.01

Regression equation	R^2	Estimate		Effect	
		Calcium (CA)	Vitamin D (VD)	Calcium (CA)	Vitamin D (VD)
BW = 208.189 + 15.9456 CA + 0.00114077 VD	0.90	-	-	Linear	Linear
WG = 123.129 + 17.3760 CA + 0.000985950 VD	0.90	-	-	Linear	Linear
FC = 3.54514 – 0.286185 CA + 0.0000415979 VD	0.86	-	-	Linear	Linear
FI = 447.196 + 0.0110283 VD	0.98	-	-	-	Linear
CW = 123.461 + 16.5786 CA + 0.00149967 VD	0.89	-	-	Linear	Linear
BrW = 52.3631 + 8.12943 CA + 0.000927708 VD	0.84	-	-	Linear	Linear
LW = 31.0242 + 2.48828 CA + 0.000459208 VD	0.66	-	-	Linear	Linear

CV - coefficient of variation; FI - feed intake; BW - body weight; WG - weight gain; FC - feed conversion; CW - carcass weight; BrW - breast weight; LW - leg weight.

Table 3 - Effect of Ca and vitamin D on bone and blood variables of quails from 15 to 35 d of age

Calcium (%)	0.42				0.58				0.74				0.90				CV (%)
Vitamin D (IU)	1,000	2,000	3,000	4,000	1,000	2,000	3,000	4,000	1,000	2,000	3,000	4,000	1,000	2,000	3,000	4,000	
BC (%)	15.51	15.35	15.91	14.77	14.58	15.58	15.46	15.65	14.75	16.03	15.85	15.33	15.20	15.90	15.44	16.15	4.10
BPH (%)	10.72	10.18	11.19	9.37	9.77	9.18	10.09	10.15	11.25	10.50	10.59	9.16	9.98	11.17	10.44	10.25	9.70
BA (%)	50.21	53.27	53.34	50.43	51.51	51.88	54.73	52.38	51.29	51.67	52.86	50.92	52.30	53.44	53.83	53.58	3.38
FBR (kgf)	6.61	6.17	7.27	6.44	6.70	7.00	5.75	6.55	7.77	6.79	6.88	7.57	6.67	6.76	7.77	7.51	11.54
TBR (kgf)	8.22	8.31	8.58	7.74	7.72	7.72	6.38	7.57	9.14	7.69	7.48	9.29	7.95	7.45	8.99	8.88	14.20
FSI (mg/mm)	12.20	12.60	12.30	13.79	14.01	13.19	13.22	12.48	14.20	13.11	12.93	13.52	13.91	13.27	13.19	13.21	6.79
TSI (mg/mm)	11.46	12.29	12.41	12.87	13.46	12.27	12.62	11.49	13.75	12.44	12.68	13.05	14.00	12.95	12.91	13.01	7.16
BD (mm Eq/Al)	1.82	2.06	1.87	1.80	1.89	1.88	1.95	1.87	1.97	2.00	1.91	1.86	1.86	1.86	2.18	2.03	13.62
BLC (%)	6.87	6.78	12.05	2.75	4.35	13.6	6.32	3.28	3.98	4.55	3.52	4.88	4.65	4.42	2.40	6.20	40.10

Regression equation	R^2	Estimate		Effect	
		Calcium (CA)	Vitamin D (VD)	Calcium (CA)	Vitamin D (VD)
BC = 14.4928 + 0.000915681 VD – 0.000000168844 VD^2	0.94	-	2,712	-	Quadratic
BA = 47.8959 + 0.00402171 VD – 0.000000744829 VD^2	0.87	-	2,700	-	Quadratic
FSI = 13.6497 + 1.39305 CA – 0.00120539 VD + 0.000000218674 VD^2	1.00	-	2,756	Linear	Quadratic
TSI = 11.3246 + 2.12773 CA	0.97	-	-	Linear	-
FBR = 5.88928 + 1.51539 CA	0.67	-	-	Linear	-
BLC = 5.86509 – 6.77668 CA + 0.00457851 VD – 0.000000976975 VD^2	0.91	-	2,343	Linear	Quadratic

CV - coefficient of variation; BC - bone calcium; BPH - bone phosphorus; BA - bone ash; FBR - femoral bone resistance; TBR - tibial bone resistance; FSI - femoral Seedor Index; TSI - tibial Seedor Index; BD - bone densitometry; BLC - blood calcium.

Some studies indicate that sexual maturity in quail occurs between 35 and 42 days of age. According to Classen and Scott (1982), calcium intake increases at slower rates before the first oviposition, which is probably a result of the high amount of calcium required to develop the medullary bone, which begins forming in response to sexual hormones.

This may explain the linear increase in calcium and vitamin D requirements in quail between 15 and 35 days of age. Keshavarz (1987) suggested an alternative method to decrease calcium requirements of medullary formation and to increase calcium storage levels in the bone. He proposed that increasing the calcium levels during the pre-oviposition period could decrease egg production, because low calcium levels could reduce the actions of follicle stimulating hormone (Taher et al., 1984).

Diets with high-calcium content yield greater amounts of available calcium in the gut and as a result, birds have enough calcium to meet normal growth standards and have enough calcium stored in the bone to utilize during the oviposition period (Vargas Jr. et al., 2004). Thus, calcium storage can be used during the oviposition period when calcium demand is high.

Leeson et al. (1986) evaluated birds that were fed diets with low-calcium content during the pre-oviposition period and observed that birds had impaired egg production when fed low-calcium diets for long periods. However, egg production normalized when calcium stores in the bone were restored.

Another factor that may be related to the linear increase of vitamin D requirements is the immunomodulatory effect of vitamin D. Klasing (1998) relayed that the immune system has a greater priority for nutrients and is able to compete with other tissues depending on the nutrient levels and the feed ingredients.

Calcium and ash contents in the bone were not affected by the increase in calcium and vitamin D levels, because they were probably being mobilized to maintain the blood calcium levels, since they were crescent, because the bones are metabolically active tissues.

The results showed that calcium levels were associated with a linear increase in diameter, length, weight, and fill of bone organic matrix because Seedor index and bone resistance were influenced by these variables.

Some authors (Alves et al., 2002; Driver et al., 2005; Bertechini, 2006) found that the reduction in dietary calcium improves the efficiency of calcium absorption due to the regulation of 1,25-Dihydroxycolecalciferol in both plasma and intestine (Morrissey and Wasserman, 1971; Montecuccolli et al., 1977). Furthermore, an increase in the concentration of duodenal calbindin, a protein found in the intestine that binds calcium from the lumen (Hunziker et al., 1982), also helps to increase absorption.

The use of calcium stores from the medullary bones to support egg production leads to a sudden loss of 2 g of body calcium. Thus, calcium stores in the bones are needed prior to the production period. Thus, significant calcium levels should be provided in the diets for the pre-oviposition period (Lesson and Summers, 2005).

Regarding calcium and vitamin D supplementation in growing meat-type quail, it can be concluded that it is not ideal to compare quail and broiler physiology or age, because quail begin to produce eggs soon after the grower stage.

Conclusions

The supplementary levels of calcium and vitamin D in meat-type quail between 15 and 35 days of age required for maximum growth and performance is greater or equal to 0.90% of calcium and equal to 4,000 IU of vitamin D; these results may be due to the greater nutrient requirements during the pre-oviposition stage.

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