

**Níveis de cálcio nas características produtivas e reprodutivas de codornas europeias**

**Dietary calcium levels on productive and reproductive traits of european quails**

**Niveles de calcio em las características productivas y reproductivas de las codornices europeas**

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**Gabriela Queiroz Faria**

ORCID: <https://orcid.org/0000-0003-3352-1331>

Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil

E-mail: [gabysqueirozfaria@gmail.com](mailto:gabysqueirozfaria@gmail.com)

**Héder José D'Ávila Lima**

ORCID: <https://orcid.org/0000-0002-8360-8227>

Universidade Federal do Mato Grosso, Brasil

E-mail: [hederdavila@yahoo.com.br](mailto:hederdavila@yahoo.com.br)

**Leonardo da Silva Costa**

ORCID: <https://orcid.org/0000-0002-1878-6094>

Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil

E-mail: [leocostajp@yahoo.com.br](mailto:leocostajp@yahoo.com.br)

**Jéssica Amaral Miranda**

ORCID: <https://orcid.org/0000-0002-6871-5462>

Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil

E-mail: [jessicamiranda@zootecnista.com.br](mailto:jessicamiranda@zootecnista.com.br)

**Graziela Maria Freitas Rocha**

ORCID: <https://orcid.org/0000-0003-2842-6428>

Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil

E-mail: [isabellibrito@gmail.com](mailto:isabellibrito@gmail.com)

**Diego Coimbra Alcantara**

ORCID: <https://orcid.org/0000-0001-5894-3317>

Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil

E-mail: [diego\\_coimbra@yahoo.com.br](mailto:diego_coimbra@yahoo.com.br)

**Isabelli Dias Brito Pereira**

ORCID: <https://orcid.org/0000-0002-4969-8791>

Universidade Federal do Mato Grosso, Brasil

E-mail: [isabellidbp@hotmail.com](mailto:isabellidbp@hotmail.com)

**Cristina Moreira Bonafé**

ORCID: <https://orcid.org/0000-0002-9508-5494>

Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil

E-mail: [cris.bonafe@yahoo.com.br](mailto:cris.bonafe@yahoo.com.br)

## Resumo

Objetivou-se com este experimento determinar o efeito de níveis de cálcio na dieta sobre as características produtivas e reprodutivas de codornas machos europeias (*Coturnix coturnix*). Aos 35 dias de idade, 60 codornas macho Europeia pesando  $248,9\text{g} \pm 8,1\text{g}$ , foram distribuídas em delineamento experimental inteiramente casualizado, com cinco níveis de cálcio (1,5%; 2,0%; 2,5%; 3,0%; 3,5%) e 12 repetições por tratamento contendo uma ave em cada repetição. O experimento foi executado desde os 35 até os 70 dias de idade. Foram avaliadas, aos 70 dias de idade, as seguintes características produtivas nos machos: consumo de ração, consumo de cálcio, ganho de peso e o peso corporal das aves. As variáveis reprodutivas analisadas foram: eclodibilidade e fertilidade dos ovos. Também foram avaliados a mortalidade embrionária precoce, intermediária e tardia. A inclusão de níveis de cálcio de 3,16% não mostrou nenhum efeito negativo sobre o desempenho produtivo de codornas e também fornece maior porcentagem de ovos eclodidos.

**Palavras-chave:** Cálcio; *Coturnix coturnix*; Fertilidade; Eclodibilidade e Desempenho.

## Abstract

The objective of this study was to determine the effect of dietary calcium levels on productive and reproductive traits of European male quail (*Coturnix coturnix*). A total of 60 European male quail, at 35 days of age, weighing  $248.9\text{g} \pm 8.1\text{g}$  were distributed in a completely randomized experimental design with five calcium levels (1.5, 2.0, 2.5, 3.0 and 3.5%), and 12 replicates per treatment (one bird each replicate). The experimental period was 35 to 70 days of age. At 70 days of age, the parameters of male productive traits assessed were: feed consumption, calcium consumption, weight gain and body weight of birds. The reproductive traits analyzed were: hatchability and fertility of eggs. Early, intermediate and late embryonic mortality were also evaluated. The diet containing 3.16% of calcium provides higher percentage of hatched eggs and did not show a negative effect on productive performance of European quails.

**Keywords:** Calcium; *Coturnix coturnix*; Fertility; Hatchability and Performance.

## Resumen

El presente estudio se realizó para determinar el efecto de los niveles de calcio en la dieta sobre las características productivas y reproductivas de la codorniz macho europea (*Coturnix coturnix*). A los 35 días, se distribuyeron 60 codornices machos europeos de  $248,9 \text{ g} \pm 8,1 \text{ g}$  en un diseño experimental totalmente aleatorizado, con cinco niveles de calcio (1,5%; 2,0%; 2,5%; 3,0%; 3,5%) y 12 repeticiones por tratamiento con un ave en cada repetición. El experimento se realizó entre los 35 y los 70 días de edad. A los 70 días de edad, se evaluaron las siguientes características productivas en los machos: ingesta de alimento, ingesta de calcio, aumento de peso y peso corporal de las aves. Las variables reproductivas analizadas fueron: incubabilidad y fertilidad de los óvulos. También se evaluó la mortalidad embrionaria temprana, intermedia y tardía. La inclusión de niveles de calcio del 3,16% no mostró ningún efecto negativo en el rendimiento productivo de las codornices y también proporciona un mayor porcentaje de huevos para incubar.

**Palabras clave:** Calcio; *Coturnix coturnix*; Fertilidad; Incubabilidad y Rendimiento.

## 1. Introdução

All animals' feed should contain the required amount of nutrients, including minerals, to express maximum productive potential. Dietary calcium regulates spermatid physiology and is involved in several stages of sperm capacitation and acrosome reaction (Kilic et al., 1996). Micro minerals are essential for enzymatic, metabolic and cellular response which reflects in reproductive hormonal functions, spermatogenesis and spermatid maturation process (Hidirolou e Knipfel, 1984).

Since the effect of calcium on muscular contraction was demonstrated, it is considered a second intracellular messenger, take part of cellular activities as cellular division, differentiation and cellular motility and also of the motility process associated with male fertility. Intracellular calcium is the main element of flagellar motility and acrosome vesicle fusion (Darszon et al., 1999). In mammals extracellular  $\text{Ca}^{2+}$  ions regulates fertilization through spermatid motility hyperactivation, acrosome reaction, oocyte spermatid fusion and oocyte activation (Suarez et al., 1993).

Several studies analyzing the relationship between calcium and reproductive traits showed controversial results. According to Hong et al., (1984), adverse effects on sperm motility have been observed not depending on calcium concentration. Nishida et al. (1996);

Heffner e Storey, (1981) e Chinoy et al. (1983) have found that small concentration of extracellular  $\text{Ca}^{2+}$  for long periods can increase fertilizing capacity and sperms motility can become insufficient over time in the absence of extracellular  $\text{Ca}^{2+}$ . Diets with a calcium deficiency decreased birds growth rate, egg production and also provides a thin eggshell (Scott et al., 1982).

There is a lack of information about calcium related to reproductive functions and productivity (egg, meat) production in European quail. The objective of this study is to determine the effects of dietary calcium levels (1.5, 2.0, 2.5, 3.0 and 3.5%) on performance and reproductive traits in European male quails.

## 2. Metodologia

The study was carried out at Federal University of the Jequitinhonha and Mucuri, Animal Science Department in Diamantina, MG. A total of 60 European male quail matched with two females per replicate were used.

Quails were reared on floor from 1 day to 35 days of age. After 35 days of age, the birds were housed in galvanized wired cages measuring 15 x 20 x 20 cm (length x width x height) in a stock density of 300  $\text{cm}^2$  per bird. Nipple drinkers and feeders were provided in each cage. Continuous light program (natural + artificial) was used, with a total of 16h of light per day.

During the experimental period the maximum and minimum temperature averages obtained by using thermometers were  $23.5 \pm 3.3$  ° C and  $20.5 \pm 1.2$  ° C, respectively.

Quails were distributed in a completely randomized experimental design with five treatments (1.5%, 2.0%, 2.5%, 3.0%, and 3.5% levels of calcium) and twelve replicates with one bird per experimental unit (cage). From 35d to 70d of age, quails were fed with a diet based on corn and soybean meal, containing 22% Crude Protein, 2800 kcal of ME/kg diet.

Experimental diets (Table 1) were formulated according to the nutritional requirements established by Rostagno et al., (2011) and Silva and Costa (2009).

Table 1: Calculated composition of experimental diets

Ingredient	Ca Level (%)				
	3.5	3.0	2.5	2.0	1.5
Corn	46.15	46.15	46.15	46.15	46.15

Soybean meal 45% CP	39.66	39.66	39.66	39.66	39.66
Soybean oil	3.80	3.80	3.80	3.80	3.80
L-Threonine (98%)	0.07	0.07	0.07	0.07	0.07
DL-Methionine (99%)	0.18	0.18	0.18	0.18	0.18
L-Lysine HCl (78.8%)	0.04	0.04	0.04	0.04	0.04
Dicalcium phosphate	1.16	0.95	0.65	0.45	0.20
Limestone	8.10	6.93	5.82	4.65	3.50
Mineral Mixture <sup>1</sup>	0.10	0.10	0.10	0.10	0.10
Vitamin Mixture <sup>2</sup>	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.54	0.54	0.54	0.54	0.54
Sand	0.10	1.48	2.89	4.26	5.66
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

#### Calculated composition

Metabolizable Energy (kcal/kg)	2800	2800	2800	2800	2800
Crude Protein (%)	22.00	22.00	22.00	22.00	22.00
Calcium (%)	3.50	3.0	2.5	2.0	1.5
Phosphorus (%)	0.32	0.28	0.23	0.19	0.14
Methionine + Cystine (%)	0.77	0.77	0.77	0.77	0.77
Lysine (%)	1.13	1.13	1.13	1.13	1.13
Fiber (%)	2.94	2.94	2.94	2.94	2.94
Sodium (%)	0.23	0.23	0.23	0.23	0.23
Threonine (%)	0.81	0.81	0.81	0.81	0.81
Tryptophan (%)	0.20	0.20	0.20	0.20	0.20
Arginine (%)	1.798	1.798	1.798	1.798	1.798
Isoleucinae(%)	1.075	1.075	1.075	1.075	1.075
Valine (%)	0.962	0.962	0.962	0.962	0.962

<sup>1</sup>Composition/Kg: manganese-160g. iron- 100g. zinc-100g. copper-20g. cobalt-2g. iodo-2g. q.s.p. – 1000g.

<sup>2</sup>Content /Kg: vit. A 12.000.000 U.I.. vit. D 3.600.000 U.I. : vit. E 3.500.000 U.I. : vit. B1 2.500.000 mg.. vit. B2 8.00.000 mg.. vit.

B6 5.000.000 000mg.. pantothenic acid 12.000 mg. biotin 200 mg. vit. K 3.000.000 mg.. folic acid 1.500 mg. nicotinic acid 40.000 mg. vit. B12 20.000.000 mg.. selenium 150 mg. q.s.p. 1.00 g.

At 70 days of age one male and two female quails were housed in a cage and fed with a diet formulated using the same levels of calcium as used in commercial farms.

Feed intake (g/quail/day) was measured in the period 35 days-70 days of age, considering mortality rate during the experiment. Calcium consumption was measured considering feed intake and calcium level on the diets.

The quails were weighed in the beginning and at the end of the experiment to obtain weight gain average. Hatchability was recorded and the number of infertile eggs was measured according to Gonzales and Cesario (2003) and Alda (2003).

Embryo mortality was recorded in the early, intermediate and late phases, characterized as below:

**Phase I- Early:** non-appearance of the “big black eye”, the presence of blastoderm remains or blood rings. The embryos dead during 48h of incubation have a cream membrane without

evident blood vessels (Gonzalez. 2005).

**Phase II - intermediate:** head positioned under the right wing, yolk in the abdomen, disruption of the air chamber (internal piping) or bark (external piping), yolk sac fully in the abdominal cavity (Gonzales. 2005).

**Phase III - Late:** head positioned under the right wing, yolk in the abdomen, breaking the tube (internal piping) or bark (external piping), yolk sac fully in the abdominal cavity (Gonzales. 2005) head.

Data were statistically analyzed using the SAS program (1990). Regression analysis were performed, and also Linear Response Plateau to select the model that fits and estimate dietary calcium levels of European male quail.

### 3. Resultados e Discussão

There was no significant effect ( $P \geq 0.05$ ) of calcium levels on feed intake and weight at 70 days of age (Table 2). However, there was a significant effect ( $P < 0.01$ ) of calcium intake in the diets containing different levels of the mineral, in the experimental diets. As there was no significant effect on feed intake, the quail receiving the diet with higher calcium level, showed a higher calcium intake. Pedroso et al. (1999) and Costa et al. (2007) also found no significant differences in feed intake with calcium levels ranging from 2.5, 3.0 and 3.5 and 2.5 % and 3.2 %, respectively, in the diet of laying quails.

Table 2: Weight gain (WG), Feed intake (FI), Calcium intake (CaI) and body weight at 70 days of age (BW70) of European male quails fed diets containing different levels of calcium

Parameter	Ca level (%)					P value
	1.5	2	2.5	3	3.5	
WG(g) <sup>NS</sup>	99.02	111.9	102	115.6	94.52	0.8519
FI(g) <sup>NS</sup>	34.03	31.17	35.4	35.36	34.68	0.407
CaI (g/quail/day) *	0.51	0.62	0.88	1.06	1.22	0.0001
BW70(g) <sup>NS</sup>	347.7	363	349.7	364.2	343.9	0.8376

\*: Linear Effect ( $P < 0.01$ );  
 NS: No significant ( $P > 0.05$ ).

There was no significant effect ( $P \geq 0.05$ ) of calcium levels on weight gain. These results are in agreement with those found by Honma (1992) and Rutz et al. (1999), which did not found influence on body weight of male breeding poultry receiving diets with calcium

levels ranging from 0.35 to 3.5%.

There was no significant effect ( $P \geq 0.05$ ) of calcium levels on reproductive parameters: intermediate and late mortality. There were significant effects ( $P < 0.01$ ) of calcium intake in the function of infertility, the rate of early mortality and hatched eggs.

Table 3: Reproductive Performance of Male European quails evaluating hatchability of eggs and stage of embryo development with five levels of calcium

Parameter	Ca level (%)				
	1.5	2.0	2.5	3.0	3.5
Infertile* (%)	42.44	47.68	38.383	22.86	20.57
Early* (%)	15.68	7.86	4.46	2.68	3.93
Intermediate <sup>NS</sup> (%)	7.30	8.09	10.72	14.11	14.86
Late <sup>NS</sup> (%)	6.44	7.44	8.53	6.79	6.29
Hatched* (%)	28.14	28.93	37.91	53.57	52.69

\*: Linear Effect ( $P < 0.05$ )

<sup>NS</sup>: No significant ( $P > 0.05$ )

Calcium is an ionic metal most abundant and important for the organism and cell physiology. Approximately 10% of calcium is in soluble form and it is involved in physiological processes such as growth process, cell locomotion, blood coagulation and fertility (Gilman et al., 1991). Dietary calcium levels are important to promote better reproductive activity.

Calcium is important for reproduction, sperm physiology, acrosome reaction, capacitation of spermatozoa (Hidiroglou and Knipfel, 1984) in order to occur oocyte fertilization. The results showed that a level of 3% of dietary calcium provides the highest percentage of hatched eggs (Tab. 3). Moustgaard (1959) indicated that an acute calcium deficiency can cause infertility in male mice. However, Rutz et al. (1999) evaluating the effect of different levels of calcium in the diet of leghorn roosters, found no relationship between calcium level and fertility of eggs.

Rutz et al. (1999) studying the semen of Leghorn roosters fed with different calcium levels, reported that birds receiving feed with lower calcium levels (0.35%) showed a better sperm vigor. The mechanisms involved in the speed of sperm movement, possibly were activated by high levels of calcium.

Rosa et al. (2011) studied the effect of different levels of calcium in the diet (0.90 % and 1.35) of Cobb 500 and reported that the lowest calcium level studied (0.90%), was sufficient to maintain the fertility, volume of semen and number of sperm cells in suitable production amounts.

The percentage of infertile eggs decreased linearly as a function of calcium levels, according to the equation  $Y = 68.6666 \pm 13.7120X$ .  $R^2 = 0.81$ . Although the percentage of embryonic mortality in early stage has decreased linearly, the model that best explained the data was the Linear Response Plateau, estimated at 2.27% calcium level which provided lower mortality in this phase according to the equation:  $\hat{Y} = 21.2620 \pm 5.7360X$ .  $R^2 = 0.74$ .

The estimated level of 3.16 % calcium in the diet of male quail showed higher percentage of hatched eggs compared to other levels, according to the equation  $Y = 17.0540 \pm 1.234X$ .  $R^2 = 0.87$ . Calcium has an influence in male quail fertility, and the calcium level of 3.16% is recommended due to high percentage of hatchable eggs.

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#### **Porcentagem de contribuição de cada autor no manuscrito**

Gabriela Queiroz Faria – 25%

Héder José D'Ávila Lima – 15%

Leonardo da Silva Costa – 10%

Jéssica Amaral Miranda – 10%

Graziela Maria Freitas Rocha – 10%

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