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Short Communication

Chlorine requirement for Japanese laying quails

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ABSTRACT - The objective of this study was to determine the chlorine nutritional requirement of Japanese Quails during the laying phase, based on performance and egg quality parameters. A total of 240 Japanese quails were distributed according to a randomized block design, with five treatments and six replicates, with 8 birds each. The experiment lasted 84 days, divided in four cycles of 21 days each. Treatments consisted of a basal diet formulated to meet the nutritional requirements, except for chlorine, and four supplementation levels with ammonium chloride, generating the levels 0.8; 1.6; 2.4; 3.2 and 4.0 g/kg. The parameters evaluated were: feed intake (g/bird/day), egg production (egg/bird/day), egg weight (g), egg mass (g), egg mass conversion (g/g), conversion per dozen eggs (kg/dz), weight of yolk (g/100 g of egg), albumen (g/100g of egg) and shell (g/100 g of egg), specific gravity (g/cm^3) and shell thickness (mm). As a result of this study, feed intake, egg weight, weights of albumen, yolk, shell and specific gravity were not affected by treatments. There was a quadratic behavior for egg production, egg mass, egg mass conversion, conversion per dozen eggs and shell thickness according to the chlorine levels in the diets, with the requirements for those parameters established at 1.54; 1.37; 1.39 and 2.78 g/kg, respectively. The recommendation is 1.39 g/kg of chlorine and 244.22 mEq/kg of electrolyte balance in diets for Japanese quails.

Key Words: *Coturnix coturnix japonica*, electrolyte balance, mineral nutrition

Introduction

Minerals are involved in part in biochemical processes (through the activation of enzyme systems) and absorption and transport of nutrients in the body (Barreto et al., 2007). They are also important in the energy transfer related to cellular metabolism in the protoplasm (phosphorus) and bone tissue constitution (calcium, phosphorus and magnesium). They contribute to establish and maintain osmotic pressure and acid-base balance (sodium, chloride and potassium) in animals (Murakami et al., 2006).

Osmoregulation is achieved by the homeostasis of these intra- and extracellular ions. Under optimal conditions, water and electrolyte contents are kept within narrow limits. However, electrolyte loss (Na^+ or K^+) without any change in body water content reduces the osmolality of these fluids (Borges et al., 2007).

Chlorine is found in cells in the extracellular fluids of the body, mainly in the form of sodium chloride and potassium chloride and in the gastric juice as hydrochloric acid. Despite its importance in birds, these mineral requirements have been little studied, perhaps because the requirements of sodium and chlorine are supplied by calcium chloride and sodium chloride (NaCl - table salt) in animal, a low-cost ingredient usually added to the feed (Murakami et al., 2006).

For the minimum amounts in their feed to allow birds to meet their nutritional requirements, it is essential that the proportion of these ions be optimal to maintain the acid-base homeostasis and to get the best performance from birds (Mongin, 1981).

The diets used in quail production are formulated based on nutritional requirements of laying hens or data described in the literature, not consistent with Brazilian conditions, which can compromise these birds productivity (Murakami & Furlan, 2002).

Due to the importance of sodium and chlorine electrolytes in quail productivity, it is necessary to make a reassessment of the nutritional requirements of quails, so that they can express all their productive potential (Pizzolante et al., 2006).

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Given the reasons above, the objective of this study was to determine the nutritional chlorine requirement for Japanese laying quails.

Material and Methods

The experiment was divided in 4 periods of 21 days. A total of 240 Japanese Quails (*Coturnix coturnix japonica*) were used at 14 weeks of age. In the 20 days prior to the experiment, quail egg production was recorded and the egg production rate in this period was calculated to obtain the uniformity of plots. At the time, the distribution of birds was determined by the body weight of quail per plot, to calculate the average initial body weight (112.7±0.8 g). Birds were separated into laying categories with average egg production of 0.853±0.51 egg/bird/day. The average egg production per treatment was 0.854, 0.855, 0.854, 0.852 and 0.850 egg/bird/day for treatments with levels 0.8, 1.6, 2.4, 3.2 and 4.0 g/kg chlorine, respectively, so the experimental period began.

Birds were housed in an experimental shed for quails, covered with clay tiles, with trough feeders and nipple drinkers, grouped in galvanized wire cages with dimensions 33 × 33 × 14 cm. Water and feed were provided *ad libitum*. The lighting program was of 17 hours, provided as natural plus artificial illumination.

The average temperature and relative humidity during the experimental period was 26.5 °C and 87%, respectively.

Diets (Table 1) were formulated to meet the requirement of quails according to the National Research Council (NRC, 1994), where the recommendations are given in total amino acids, because at the time of the study evaluation, there were no recommendations for digestible amino acids.

The electrolyte balance of experimental diets (EBD) was calculated according to Mongin (1980), using the formula: EBD = $\%Na^+ \times 10000/22.900^* + \%K^+ \times 10000/39.102^* - \%Cl^- \times 10000/35.453^*$, in which * are the gram equivalent Na^+, K^+ and Cl^-, respectively).

The variables studied were: feed intake (g/bird/day), egg production (egg/bird/day), egg weight (g), egg mass (g/bird/day), feed conversion ratio (kg/kg and kg/dozen), yolk (g/100g of egg), albumen (g/100g of egg) and shell (g/100g of egg) weights, specific gravity (g/cm^3) and shell thickness (mm). For this analysis, two eggs per plot were collected over the last three days of each period to weight each component and two eggs to determine the specific gravity.

Feed intake was determined from the weight difference obtained between the amounts of feed provided at the beginning and leftovers at the end of each cycle of 21 days, adjusted according to bird mortality. Egg production was calculated by the ratio of number of eggs produced by the number of birds housed in the period, multiplying the value by one hundred. Feed conversion was calculated in two ways: by dividing the feed intake (kg/bird) by the number of eggs dozens produced (dz/bird) and dividing the feed intake (kg/bird) by the egg mass produced (kg/bird), corrected for bird mortality. The egg mass in g/bird/day was calculated by multiplying production by the eggs weight.

Eggs were weighed individually in a digital three-digit scale (0.001g) (Shimadzu, model BL-320H) and the values obtained were used for the calculation of the average egg

Table 1 - Ingredients and calculated composition of experimental diets

Item	Levels of chlorine (g/kg)				
	0.8	1.6	2.4	3.2	4.0
Corn	506.18	506.18	506.18	506.18	506.18
Soybean meal, 45%	380.86	380.86	380.86	380.86	380.86
Dicalcium phosphate	12.82	12.82	12.82	12.82	12.82
Limestone	53.26	53.26	53.26	53.26	53.26
Soybean oil	33.58	33.58	33.58	33.58	33.58
Sodium bicarbonate	3.75	3.75	3.75	3.75	3.75
DL-methionine	1.27	1.27	1.27	1.27	1.27
Ammonium chloride	0.00	1.21	2.42	3.63	4.83
Salt	0.18	0.18	0.18	0.18	0.18
Choline chloride	1.00	1.00	1.00	1.00	1.00
Mineral supplement[1]	1.00	1.00	1.00	1.00	1.00
Vitamin supplement[2]	1.00	1.00	1.00	1.00	1.00
Antioxidant[3]	0.10	0.10	0.10	0.10	0.10
Inert[4]	5.00	3.79	2.58	1.37	0.17
Calculated chemical composition					
Metabolizable energy (kcal/kg)	2,900	2,900	2,900	2,900	2,900
Crude protein (g/kg)	200.0	200.0	200.0	200.0	200.0
Calcium (g/kg)	25.00	25.00	25.00	25.00	25.00
Available phosphorus (g/kg)	3.50	3.50	3.50	3.50	3.50
Chlorine (g/kg)	0.80	1.60	2.40	3.20	4.00
Sodium (g/kg)	1.50	1.50	1.50	1.50	1.50
Potassium (g/kg)	8.53	8.53	8.53	8.53	8.53
Total lysine (g/kg)	10.0	10.0	10.0	10.0	10.0
Methionine + cystine (g/kg)	7.00	7.00	7.00	7.00	7.00
Methionine (g/kg)	4.34	4.34	4.34	4.34	4.34
Threonine (g/kg)	7.40	7.40	7.40	7.40	7.40
Tryptophan (g/kg)	2.55	2.55	2.55	2.55	2.55
Glycine + serine (g/kg)	19.73	19.73	19.73	19.73	19.73
Electrolyte balance (mEq/kg)	260.83	238.27	215.70	193.14	170.57
(Na + K)/Cl	12.54	6.27	4.18	3.13	2.51

[1] Mineral supplement per kg of diet: Mn - 60 g; Fe - 80 g; Zn - 50 g; Cu - 10 g; Co - 2 g; I - 1 g; excipient q.s. - 500 g.
[2] Vitamin supplement per kg of diet: vit. A - 15,000,000 IU; vit. D3 - 1,500,000 IU; vit. E - 15,000 IU; vit. B1 - 2.0 g; vit. B2 - 4.0 g; vit. B6 - 3.0 g; vit. B12 - 0.015 g; nicotinic acid - 25 g; pantothenic acid - 10 g; vit. K3 - 3.0 g; folic acid - 1.0 g; bacitracin zinc - 10 g; selenium - 250 mg.
[3] Antioxidant (BHT – butylated hydroxytoluene) - 10 g, or excipient. q.s. - 1,000 g.
[4] Washed sand.

weight. Yolks and albumens were weighed on the same scale and values were used in the calculation to obtain the percentage of each component. The percentage was determined by the ratio between the average weight of yolk and albumen and the average egg weight and the result was multiplied by 100, thus expressing the percentage.

Shells of broken eggs were identified and incubated at 55-60 °C for 24 hours for drying to determine the internal quality. After these procedures, shells were weighed on a 0.001 g accurate digital scale to obtain shell average weight.

Egg specific gravity was determined by flotation in saline solution, according to the methodology described by Hamilton (1982). Eggs were immersed in fifteen different sodium chloride solutions (NaCl) at densities ranging from 1.065 to 1.100 g/cm³ with gradient of 0.0025 g/cm³ between them. The density of the solutions was routinely checked by an oil hydrometer (Incoterm, model 5582). Skin thickness was obtained through the use of digital micrometer (Mitutoyo, model 293/230) with an accuracy of 0.001 mm after being dried at 55 – 60 °C for 24 hours.

Statistical analyses were performed using the computational package SAEG (System for Statistical and Genetics Analysis, version 8.0), and the chlorine requirement was established by linear and quadratic regression models.

Results and Discussion

There was no significant effect (P>0.05) of chlorine levels on feed intake or egg weight; however, the variables egg production, egg mass, feed conversion per egg mass and dozen eggs showed significant effect (P<0.01) (Table 2).

Just as in the present study, the absence of significant effects (P>0.05) of chlorine levels on feed intake were also obtained by Rodrigues et al. (2008) and Costa et al. (2008), who studied the chlorine requirements for Japanese quails from 1 to 21 days and 22 to 42 days of age, respectively. Judice et al. (2002) found significant differences between cationic and anionic diets noting an increased consumption in cationic diets. In this experiment, the anionic diets may have inhibited the consumption due to high chlorine content in the diet (8.4 g/kg).

Ribeiro et al. (2007), studying the sodium and chlorine requirements of Japanese quails (65 days old), using the levels 0.8, 1.8, 2.8 and 3.8 g/kg chlorine, also observed no significant effect of this element levels on feed intake. However, these researchers observed a quadratic effect of chloride levels on egg production, egg weight and feed conversion per egg mass and specific gravity, indicating an improvement in performance with increasing chlorine above 0.8 g/kg and worsening in levels above 2.5 and 2.6 g/kg for egg production and feed conversion per egg mass, respectively.

Still according to Ribeiro et al. (2007), low or high dietary levels of chloride in the diet can lead to alkalosis and metabolic acidosis, respectively; these effects are likely responsible for the drop in quail performance at extreme chlorine levels in the diets, since these researchers observed that there will be changes in the acid-base balance in the body of the birds.

Diets formulated with high Cl contents (NH^4Cl, HCl and $CaCl^2$) decrease blood pH in broilers, adversely affecting their growth under thermoneutrality. However, acid or base intake, electrolyte balance, the environment, their interactions and implications on the performance of birds still have to be defined (Borges et al., 2007).

Sodium chlorine is an essential mineral for hens. A low-salt diet causes a significant depression in egg weight and a reduction in feed intake (Persson, 2009).These levels

Table 2 - Effect of different levels of chlorine in the diet for Japanese quails on feed intake, egg production, average egg weight, egg mass, egg mass conversion and conversion per dozen eggs

Chlorine levels (g/kg)	Feed intake (g/bird/day)	Egg production[1] (egg/bird/day)	Average egg weight (g)	Egg mass[2] (g/bird/day)	Egg mass conversion[3] (g/g)	Conversion per dozen eggs[4] (kg/dz)
0.8	27.3	0.933	13.2	12.3	2.2	0.35
1.6	26.0	0.939	13.6	12.7	2.0	0.33
2.4	27.4	0.921	13.8	12.1	2.3	0.40
3.2	26.6	0.902	12.8	11.5	2.3	0.35
4.0	26.4	0.829	12.9	10.7	2.5	0.40
P value						
Linear	0.275	0.001	0.112	0.001	0.001	0.001
Quadratic	0.980	0.001	0.602	0.039	0.037	0.003
Lack of fit	0.038	0.337	0.321	0.627	0.115	0.022
CV (%)	2.77	1.82	3.86	4.92	4.95	2.66

CV - coefficient of variation.
[1] $\hat{Y} = -0.0177x^2 + 0.0546x + 0.8988$ ($R^2 = 0.98$).
[2] $\hat{Y} = -0.2679x^2 + 0.7357x + 11.98$ ($R^2 = 0.97$).
[3] $\hat{Y} = 0.0558x^2 - 0.1554x + 2.24$ ($R^2 = 0.95$).
[4] $\hat{Y} = 0.0074x^2 - 0.0251x + 0.3637$ ($R^2 = 0.81$).

of chlorine were also studied by Rachel et al. (2010) with Italian quails, and the authors did not observe significance effects on the performance of these birds in the phase from one to 49 days old.

In the literature, it can be verified that the effects of chlorine levels on the performance parameters of laying hens have also been variable, with negative linear effect on feed conversion with increasing chlorine levels reported by Murakami et al. (2001), when studying the sodium and chlorine requirements for layers. Faria et al. (2000) found no significant differences in the performance of hens fed a diet containing 1.6 or 2.2 g/kg chlorine, 1.6 g/kg sodium and 2.2 g/kg potassium. It is noteworthy that these studies did not report the mechanisms involved in the chlorine effects on performance of laying hens.

In relation to egg weight, the results from this study are consistent with Junqueira et al. (2000), who observed the effect of using different sources of sodium (Na), chlorine (Cl) and potassium (K) as well as levels of these electrolytes in the diet and (Na + K)/Cl ratio for Hy-Line white hens at 54 weeks of age, which presented better egg weight with the ratios of 3.46 and 4.46, using the levels 2.0 and 2.6 g/kg of chlorine, respectively.

Costa et al. (2008), studying the chlorine requirement of growing Japanese quails from 22 to 42 days of age and its effect on the initial egg production, observed quadratic effect of chloride levels on egg production ($P<0.01$); these authors obtained maximum point of 2.1 g/kg chlorine (Table 2). However, Junqueira et al. (2000) observed no differences ($P>0.05$) in egg production and feed intake of hens fed diets containing different ratios of (Na + K)/Cl.

In this study, the egg mass was better when the birds were fed the diet with 1.37 g/kg chlorine (Table 2). Murakami et al. (2006), studying the determination of the best level of common salt for Japanese quails at 13 weeks of age, evaluated seven treatments (0.0, 1.5, 2.0. 2.5, 3.0, 3.5 and 4.5 g/kg common salt) and observed a significant effect ($P<0.05$) on egg production, feed intake, feed conversion, average egg weight, egg mass and shell thickness. These researchers concluded that birds showed better performance and egg shell quality with 1.5 g/kg common salt (equivalent to 1.0 and 1.2 g/kg of sodium and chlorine, respectively).

The feed conversion per egg mass showed statistical difference between chlorine levels; the diet with 4.0 g/kg chlorine showed the worst results. In both conversions, the diet that showed the best results was that containing up to 1.39 g/kg chlorine. Likewise, Murakami et al. (2003) found an estimated 1.8 g/kg Cl for the feed conversion per egg mass in Leghorn hens.

Leeson & Summers (2001) recommend that the chlorine levels on a diet for birds should exceed 10 to 15% the Na levels. The optimal level observed in this experiment for feed conversion per dozen eggs was obtained at 13% Cl excess compared with Na, agreeing with this recommendation.

The points of maximum egg production (1.54 g/kg), egg mass (1.37 g/kg), egg mass conversion (1.39 g/kg) and conversion per dozen eggs (1.69 g/kg) were estimated by the derivative of regression equations (Table 2).

Of the variables evaluated for egg quality of Japanese quails, only the shell thickness was influenced by treatments with different levels of chlorine (Table 3).

Another variable that showed significant effects in relation to chlorine levels in the diet was shell thickness, presenting the best levels in those diets containing 2.4 and 3.2 g/kg of this mineral, with the best shell thickness estimated at 2.78 g/kg chlorine, representing 204.42 mEq/kg.

Chen & Balnave (2001) claim that the carbonic anhydrase, enzyme directly related to shell formation, has optimal activity at slightly alkaline pH and that, moreover, excess chlorine intake limits calcium transport to the uterus and the bicarbonate concentration in the lumen, affecting the shell egg quality.

Table 3 - Effect of different levels of chlorine in diets for Japanese quails on weight of albumen, yolk and shell, specific gravity and shell thickness

Chlorine levels (g/kg)	Albumen (g/100 g of egg)	Yolk (g/100 g of egg)	Shell (g/100 g of egg)	Specific gravity (g/cm^3)	Shell thickness (mm)[1]
0.8	58.0	29.2	7.8	1.1	0.466
1.6	57.0	30.3	7.8	1.1	0.489
2.4	56.8	29.6	8.0	1.1	0.492
3.2	59.4	28.1	8.0	1.1	0.493
4.0	57.0	30.1	7.7	1.1	0.485
P value					
Linear	0.920	0.844	0.936	0.307	0.067
Quadratic	0.976	0.731	0.052	0.075	0.024
Lack of fit	0.043	0.129	0.302	0.459	0.806
CV (%)	2.71	5.40	2.19	0.08	2.77

CV - coefficient of variation.
[1] $\hat{Y} = -0.0071x^2 + 0.0395x + 0.4404$ ($R^2 = 0.96$).

Concerning the values of albumen weight, yolk and shell, specific gravity, and shell thickness, it can be observed that no significant differences between treatments were present. However, Murakami et al. (2003) found that the increase in chlorine levels improved the shell percentage and specific gravity, since the Na levels were lower than those of chlorine in diets for Leghorn hens. In turn, Faria et al. (2000) observed shell quality improvement with chlorine reduction from 2.2 to 1.6 g/kg in diets for laying hens.

Studying the chlorine requirements for quails, Ribeiro et al. (2007) observed a quadratic effect of chlorine levels in diets on eggs specific gravity, which increased up to the estimated level of 2.1 g/kg chlorine, worsening at levels above this. According to the researchers, the improvement in shell quality associated to chlorine is due to bolus acidification, increasing the solubility and absorption of minerals like calcium and phosphorus, which are vital for the strength and quality of egg shells.

Conclusions

The recommended levels of chlorine and electrolytic balance in diets for better performance of Japanese quails are 1.39 g/kg and 244.22 mEq/kg, respectively.

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