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# Physiological variables and quality of quail eggs supplemented with magnesium in water under thermal stress

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**Abstract**

The objective of this study was to evaluate the effects of magnesium supplementation in the water of Japanese quails (*Coturnix coturnix japonica*) on physiological variables and egg quality with the birds kept in a thermoneutral environment and under thermal stress. A total of 394 quails were used, distributed in a completely randomized 2×4 factorial design (two temperatures: 24 and 32°C, and four levels of magnesium: 50, 150, 250, and 350 mg $L^{-1}$), with six replicates and eight birds per experimental unit. The respiratory rate ($P < 0.0001$), head temperature ($P < 0.0001$), wing temperature ($P < 0.0001$), foot temperature ($P < 0.0001$), back temperature ($P < 0.0001$), body surface temperature ($P < 0.0001$), and average body temperature ($P < 0.0001$) showed significant differences between the temperatures tested. With the exception of head temperature (0.0045), the other variables did not change their values with the increase in the level of magnesium in the water. Egg mass showed a significant difference (0.0072) as a function of the level of magnesium in the water. The temperature of the head was higher in birds consuming water with higher levels of magnesium, causing an increase in respiratory rate, and head, wing, foot, back, average surface, and body temperatures in birds under thermal stress.

**Keywords:** ambience; *Coturnix*; quail farming; avian physiology.

**Practical Application:** Supplementation with magnesium oxide and Mg protein promoted an increase in weight gain and feed consumption of Japanese quails kept under cyclic temperatures of thermal stress.

## 1 INTRODUCTION

Supplying magnesium in water or feed can minimize the severity of heat stress in broilers and laying birds and reduce its effects on physiological variables, egg production, and quality (Shastak & Rodehutscord, 2015; Yang et al., 2012). Poultry can receive magnesium levels in the water of up to 150 mg $L^{-1}$ (Shastak & Rodehutscord, 2015). Above this limit, adverse effects such as intestinal irritation, diarrhea, and reduction in the rate of growth, production, and quality of eggs may occur (Kim et al., 2013). Supplementation with magnesium in the diet of laying hens provided an increase in egg weight and quality (Seo et al., 2010; Kim et al., 2013).

Supplementation with magnesium oxide and Mg protein promoted an increase in weight gain and feed consumption of Japanese quails kept under cyclic temperatures of thermal stress (Tao et al., 1983). Laying quails raised in high temperatures may have a reduction in their productive performance and egg quality (El-Tarabany et al., 2016; Silva et al., 2017; Soares et al., 2021a), and to maintain homeostasis, quails alter their ingestive behavior (Castro et al., 2017; Soares et al., 2021b) and raise the rectal and surface temperatures, and the heart and respiratory rates (Rodrigues et al., 2016).

In environments with high temperatures, quails raise cloacal and surface temperatures to cool the body core and facilitate the exchange of sensitive heat with the environment (Rodrigues et al., 2016), increase the heart rate, and stimulate blood flow to peripheral tissues not covered with feathers (feet and facial area) and highly vascularized body regions such as ridges and dewlap (Santos et al., 2019), which have membranous surfaces and a rich vascular network, making these regions important thermolysis sites.

The main hypothesis of this study is that magnesium supplementation in the water offered to quails reduces the effects of thermal stress on birds reared in warm environments. Therefore, the objective of this research was to evaluate the influence of increasing levels of magnesium in the water on the physiological variables and the quality of the eggs of Japanese quails kept at a thermoneutral temperature and under thermal stress.

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## 2 MATERIALS AND METHODS

### *2.1 Ethics Committee and experimental site*

Research on animals was conducted according to the institutional committee on animal use (Protocol Number 089.2017).

The experiment was carried out in the Campina Grande, municipality of Campina Grande, Paraíba, Brazil (7º13'11"S; 35º53'31"O and 547 m altitude). According to the Köppen climate classification, the climate of the region is classified as tropical, with wet and dry seasons (AS'), with a maximum annual temperature of 32°C, a minimum of 17°C, and an average annual rainfall of 765 mm.

### *2.2 Climatic chambers*

The experiment was carried out in two climatic chambers, with dimensions of 3.07 m length × 2.77 m width × 2.6 m height, located at the Rural Construction and Ambience Laboratory at UFCG.

For environmental control, the chambers were equipped with an electric resistance air heater, a hot/cold split air conditioner, with a power of 18,000 btus, and an air humidifier, with a capacity of 4.5 L and a fog flow (average value) of 300 mL $h^{-1}$. The relative humidity of the air was controlled by air humidifiers and measured by sensors. The wind speed was obtained through side fans and exhaust fans. The chambers had temperature and humidity sensors, and environmental data were collected and recorded every 15 min by sensors coupled to the data acquisition system, through a controller of the type MT-530 PLUS *Full Gauge Controls*®, controlled via computer through SITRAD® (software for data acquisition, control, monitoring, and visualization in climatic chambers).

Fluorescent lamps of 20 W and 220 V were used to light the chambers, and the daily light program that was adopted was of 17 h of light and 7 h of darkness. From 7 p.m. onward, the doors were opened and remained until 7 a.m. the following day at room temperature (22 ± 2.0°C), simulating the environmental conditions of semiarid regions. The relative air humidity in the chambers during the experimental period was 65.0 ± 5.0%, and the average wind speed was 2.0 ± 0.5 m $s^{-1}$.

### *2.3 Animals and management and experimental design*

In the pre-experimental period, 394 9-week-old quails were used, with the average weight of birds being 168 ± 5 g at the beginning of the experiment (14 weeks of age) and 175 ± 5 g at the end (24 weeks of age). Housed in clusters of cages in the chambers, each cluster consisting of four floors, three cages per floor, made of galvanized wire with dimensions 50 × 33 × 20 cm (width, depth, and height, respectively). Submitted to the stocking rate of 206 $cm^2$ $bird^{-1}$, 10 birds per cage in the first phase and eight birds per cage in the second phase. The cages were equipped with zinc sheet trough feeders and individual nipple drinkers.

The birds went through an adaptation period of 3 weeks, in which the chambers were programmed to keep the quails under thermal comfort temperature (24°C) during the day and at room temperature at night. Egg production was counted, and at the end, the quails were weighed for homogeneous distribution in the experimental units, considering the body weight and the average laying rate of the birds. After the distribution, the chamber temperatures were adjusted to 24.0 ± 1.0°C, within the thermal comfort zone and 32 ± 1.2°C, above the thermal comfort zone (Castro et al., 2017; Soares et al., 2021b). These values were maintained for a period of 12 h (7 a.m. to 7 p.m.). The chamber doors were opened from 7:01 p.m. to 6:59 a.m. at room temperature, simulating the conditions of the Brazilian semiarid.

For the experimental period, 384 14-week-old quails were selected and distributed in a completely randomized experimental design, in a 2 × 4 factorial scheme (two temperatures (24 and 32°C) × four levels of magnesium in the water (50, 150, 250, and 350 mg $L^{-1}$)), totaling eight treatments

### *2.4 Drinking water: preparing and management*

Four levels of magnesium were used in water 50, 150, 250, and 350 mg $L^{-1}$. To obtain these levels, magnesium chloride ($MgCl_2 \cdot 6H_2O$), PA with 203.3 molecular weight of salt was added to water mineral with low salt content (1.45 mg $L^{-1}$ Mg) considering and correcting to obtain total treatment weight (Tn), according to Equation 1:

$$MgCl_{2Tn} = (Mg_{Tn} - Mg_a)/0.12 \quad (1)$$

where:

$MgCl_{2Tn}$: magnesium chloride salt to be added to the water in mg $L^{-1}$;

$Mg_{Tn}$: magnesium desired in treatment in mg $L^{-1}$;

$Mg_a$: magnesium contained in water based on mg $L^{-1}$.

In the composition of the salt, as the magnesium chloride was hexahydrate, the weight of magnesium in the composition represented 12% of the total weight of added salt. The remainder was composed of chloride ions (Table 1) and water molecules.

The waters with magnesium concentrations were stored in 200-L buckets with lids and used daily in the drinkers according to the treatments. The water was prepared by weighing the proportion of MgCl2 salt for each 10 L of water for each treatment, and the electrical conductivity level was checked at completion of each prepared dilution by using a digital conductivity meter (model ITCD – 1000 Instrutemp). Water analysis was carried out at the UFCG Irrigation and Salinity Laboratory (Table 1).

### *2.5 Diet*

During the experimental period, the birds were subjected to identical feed management, consuming feed for laying quails based on corn and soybean meal. The nutritional composition of the ingredients used was obtained based on the tables by Rostagno et al. (2011), with water and feed being provided daily, manually and *ad libitum*. Leftovers and waste were weighed and discounted from the amount of feed weighed initially to calculate the feed and water intake of the birds.

**Table 1**. Water already added with magnesium chloride ($MgCl_2$).

| Variable | Magnesium chloride | | | |
|---|---|---|---|---|
| | 50 | 150 | 250 | 350 |
| Ph | 7.70 | 7.70 | 7.70 | 7.20 |
| Electric conductivity (uS $cm^{-1}$) | 737.50 | 2,016.00 | 3,219.00 | 4,204.00 |
| Calcium (mg $L^{-1}$) | 4.00 | 4.80 | 7.00 | 5.60 |
| Magnesium (mg $L^{-1}$) | 50.00 | 150.00 | 250.00 | 350.00 |
| Sodium (mg $L^{-1}$) | 92.68 | 97.06 | 94.76 | 94.76 |
| Potassium (mg $L^{-1}$) | 0.78 | 0.78 | 0.78 | 0.78 |
| Chlorides (mg $L^{-1}$) | 173.70 | 492.05 | 866.75 | 1,152.00 |
| Bicarbonates (mg $L^{-1}$) | 42.70 | 42.09 | 41.48 | 43.31 |
| Carbonates (mg $L^{-1}$) | – | – | – | – |
| Iron (mg $L^{-1}$) | 0.07 | 0.07 | 0.07 | 0.07 |

### *2.6 Variables the production*

The variables were evaluated in three periods of 21 days, and at the end of each period, four eggs were separated per plot, two to determine the weight and percentage of yolk, albumen and shell, and two to obtain the shell thickness (Guimarães et al., 2014) and specific gravity (Alleoni & Antunes, 2001). To determine the weight and percentage of yolk, albumen, and shell, manual separation of the components was carried out, and then they were weighed individually on a digital electronic scale (0.001 g). The values obtained were used in the calculation of the percentage.

The shells were identified and kept in an oven at 105°C for 4 h to dry. After 30 min of cooling, they were weighed on a digital electronic scale (0.001 g) to obtain the average shell weight. To obtain this parameter, the weight of the dry shell was divided by the weight of the whole egg and then multiplied by 100.

The specific gravity analysis was determined and measured by means of an Incoterm brand (OM-5565®) oil densimeter, by the method of saline fluctuation (Hamilton, 1982). The eggs were immersed and evaluated in saline solutions of NaCl, with the necessary adjustments for a volume of 25 L of water with densities ranging from 1.070 to 1.090 with an interval of 0.0025. The shell thickness was determined after the egg was broken in the middle (equatorial region), dried in an oven at 105°C for a period of 4 h, and measured by using a 0–150 mm digital pachymeter, with an accuracy of 0.001 mm.

### *2.7 Variables physiological*

Twice a week, the physiological variables were measured once inside the climatic chamber: respiratory rate (RR), cloacal temperature (CT) and body surface temperature (BST), 2 h after feeding, to avoid interference of caloric increase. Two birds from each plot were used and then marked and identified.

The respiratory rate (mov $min^{-1}$) was obtained through visual evaluation, considering the number of times the birds inhaled air during 20 s, and later the value obtained was multiplied by three; for cloacal temperature (°C), a digital veterinary clinical thermometer was inserted about 2 cm into the birds' cloaca for an average of 2 min or until the temperature stabilized (Bonfim et al., 2016); for surface and average temperatures, an infrared thermometer with laser sight (-10 to 50°C) was used to measure the temperature of the head, wing, foot, and back, from a distance of 10 cm between the animal and the equipment, calculating the average surface temperature (AST) (Equation 2), according to the equation proposed by Nascimento et al. (2011). From the result, a second equation proposed by Richards (1971) was used to predict the mean body surface temperature (BT) (Equation 3):

$$\mathbf{AST} = (0.03 * T\ head) + (0.70 * T\ torso) + (0.12 * T\ wing) + (0.15 * T\ foot) \quad (2)$$

$$\mathbf{BST} = (0.3.AST) + (0.7.T\ cloacal) \quad (3)$$

### *2.8 Statistical analysis*

The means were compared by Tukey's test at 5% probability using the general linear model (GLM) procedure, and regression analysis was undertaken using the REG procedure of SAS (Statistical Analysis System, 2008).

The following mathematical model was used (Equation 4):

$$Yijk = \mu + Zi + \beta j + Z\beta ij + \varepsilon ijk \quad (4)$$

where:

Yijk: the dependent variable;

μ: the overall mean;

Zi: the effect of the i level of magnesium in water (i = 50, 150, 250, and 350 mg $L^{-1}$);

βj: the effect of level j on temperature β (j = 24 and 32°C);

Zβij: the effect of the interaction between the i level of magnesium in the water and the j level of the temperature tested;

Ɛijk: the random error, considering mean 0 and variance $\sigma^2$.

## RESULTS

The RR ($P < 0.0001$), HT ($P < 0.0001$), WT ($P < 0.0001$), FT ($P < 0.0001$), BT ($P < 0.0001$), BST ($P < 0.0001$), and ABT ($P < 0.0001$) showed a significant difference between the temperatures tested, and all significant variables had higher values at a temperature of 32°C (Table 2). With the exception of Ht ($P = 0.0045$), the other variables do not change their values with the increase in the level of magnesium in the water (Table 2). The levels of 150 and 350 mg $L^{-1}$ of magnesium in water have the highest values for HT and statistically similar to the level of 250 mg$L^{-1}$. The variables did not show an interaction effect between temperature and magnesium ($P > 0.05$) (Table 2).

The egg quality variables showed no significant difference ($P > 0.05$) as a function of the temperatures tested (Table 3). Egg mass showed a significant difference (0.0072) as a function of the level of magnesium in the water (Table 3). Egg mass presented the highest value at the level of 250 mg $L^{-1}$ of magnesium in water, being statistically similar to the levels of 50 and 150 mg $L^{-1}$, whereas at the level of 350 mg $L^{-1}$, it presented a lower value for egg mass. Egg quality variables showed no interaction ($P > 0.05$) between magnesium and temperature. Egg mass and thickness showed a quadratic regressive effect (Table 3).

## 4 DISCUSSION

The largest percentage of magnesium ingested, absorbed, and metabolized in the body of birds is fixed in the bones, and small amounts are found in the molecular mass and blood (Shastak & Rodehutscord, 2015). The increasing levels of magnesium in the water did not change the respiratory rate of the quails, which was within the physiological limits for species from 20 to 30 mov $min^{-1}$ (Ribeiro et al., 2016; Rodrigues et al., 2016). Maintaining respiratory rate maintains blood pH, preventing respiratory alkalosis and carbon dioxide losses, which can affect partial pressure, leading to a drop in the concentration of carbonic acid and hydrogen, and affecting the electrolyte balance of birds (Khalilipour et al., 2019). Even with the highest amount of magnesium in the water, there was no change in the feces and urine of the birds, which were within normal limits.

The increase in air temperature can change the physiological variables of birds (Rodrigues et al., 2016), and the increase in the respiratory rate of quails at a temperature of 32°C is a way to stimulate heat loss by latent mechanisms, used as mechanism for maintaining body temperature (Ribeiro et al., 2016; Rodrigues et al., 2016). However, sudden increases in respiratory rate or long periods can cause dehydration and reduce quail egg production and quality (Ribeiro et al., 2016).

**Table 2**. Averages of the physiological parameters of Japanese quails subjected to different levels of magnesium in the water, at comfort temperature (24°C) and heat stress temperature (32°C).

| Effect | | RR | CT | HT | WT | FT | BT | BST | ABT |
|---|---|---|---|---|---|---|---|---|---|
| Magnesium (M) (mg $L^{-1}$) | 50 | 24.1 ± 1.7 a | 41.9 ± 0.2 a | 33.53 ± 1.2 b | 34.6 ± 1.4 a | 29.7 ± 2.7 a | 30.7 ± 2.3 a | 31.1 ± 2.2 a | 38.7 ± 0.7 a |
| | 150 | 25.0 ± 2.0 a | 41.9 ± 0.2 a | 34.14 ± 1.3 a | 34.1 ± 2.0 a | 29.6 ± 2.9 a | 30.0 ± 2.9 a | 30.5 ± 2.7 a | 38.5 ± 0.9 a |
| | 250 | 24.3 ± 1.6 a | 41.9± 0.2 a | 34.01 ± 1.6 ab | 34.2 ± 1.8 a | 29.4 ± 3.2 a | 30.8 ± 2.6 a | 31.1 ± 2.5 a | 38.6 ± 0.8 a |
| | 350 | 23.9 ± 1.8 a | 41.9 ± 0.2 a | 34.28 ± 1.7 a | 34.6 ± 1.3 a | 30.1 ± 2.8 a | 30.6 ± 2.5 a | 31.1 ± 2.3 a | 38.7 ± 0.7 a |
| Temperature (T) (°C) | 24°C | 22.9 ± 1.1 B | 41.8 ± 0.2 A | 32.7 ± 0.4 B | 33.0 ± 1.0 B | 27.0 ± 1.0 B | 28.2 ± 0.9 B | 28.7 ± 0.8 B | 37.9 ± 0.3 B |
| | 32°C | 25.8 ± 1.0 A | 41.9 ± 0.2 A | 35.3 ± 0.7 A | 35.8 ± 0.6 A | 32.4 ± 0.7 A | 32.9 ± 0.8 A | 33.2 ± 0.7 A | 39.3 ± 0.3 A |
| *P-value* | T | < 0.0001 | 0.1479 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | M | 0.0874 | 0.9851 | 0.0045 | 0.3028 | 0.3648 | 0.0966 | 0.2072 | 0.4319 |
| | Tx M | 0.6990 | 0.8189 | 0.0647 | 0.4531 | 0.5744 | 0.5853 | 0.6054 | 0.9271 |

[A,B]Average temperatures followed by the same capital letter in the columns are statistically equal by the Tukey's test at 5% probability;

[ab]Averages of magnesium content in water followed by the same lowercase letter in the columns are statistically equal by the Tukey's test at 5% probability; RR: Respiratory rate; CT: cloacal temperature; HT: head temperature; WT: wing temperature; FT: foot temperature; BT: back temperature; BST: body surface temperature; ABT: average body temperature.

**Table 3**. Average of the characteristics of Japanese quail eggs subjected to different levels of magnesium in the water at comfort temperature (24°C) and heat stress temperature (32°C).

| Effect | | Albumen (%) | Egg yolk[1] (%) | Bark (%) | Thickness[2] (μm) | Specific gravity |
|---|---|---|---|---|---|---|
| Magnesium (M) (mg $L^{-1}$) | 50 | 55.7 ± 1.2 a | 35.7 ± 1.8 ab | 8.4 ± 0.3 a | 353 ± 18 a | 1.0739 ± 0.0008 a |
| | 150 | 55.9 ± 1.1 a | 35.8 ± 1.2 ab | 8.3 ± 0.3 a | 347 ± 30 a | 1.0740 ± 0.0020 a |
| | 250 | 55.4 ± 1.4 a | 36.7 ± 1.4 a | 8.2 ± 0.3 a | 337 ± 23 a | 1.0740 ± 0.0015 a |
| | 350 | 56.4 ± 0.7 a | 35.2 ± 0.7 b | 8.3 ± 0.3 a | 358 ± 30 a | 1.0735 ± 0.0012 a |
| Temperature (T) (°C) | 24 °C | 55.9 ± 1.1 A | 35.7 ± 1.1 A | 8.3 ± 0.3 A | 348 ± 23 A | 1.0741 ± 0.0014 A |
| | 32 °C | 55.8 ± 1.1 A | 36.0 ± 1.4 A | 8.2 ± 0.3A | 350 ± 30 A | 1.0736 ± 0.0014 A |
| *P-value* | T | 0.9413 | 0.1116 | 0.4355 | 0.7989 | 0.1629 |
| | M | 0.4056 | 0.0072 | 0.9780 | 0.0594 | 0.6745 |
| | TxM | 0.8410 | 0.1090 | 0.5917 | 0.1789 | 0.4065 |

[A,B]Average temperatures followed by the same capital letter in the columns are statistically equal by the Tukey's test at 5% probability; [ab]Averages of magnesium content in water followed by the same lowercase letter in the columns are statistically equal by the Tukey's test at 5% probability; $Y^1 = 34.61 + 0.026x - 0.00007x^2$ ($R^2 = 0.59$); $Y^2 = 366.31 - 0.265x + 0.0007x^2$ ($R^2 = 0.75$)

The average cloacal temperature of the birds (41.9°C) was not affected by the levels of magnesium in the water, remaining within the normal range for species, which can range from 41 to 42°C for adult birds (Ribeiro et al., 2016; Soares et al., 2021b). Even when kept in environments considered above their thermal comfort zone, quails maintained their homeotherm, demonstrating their adaptability to hot climates (Rodrigues et al., 2016; Silva et al., 2017). Akdemir et al. (2019) mentioned that egg production and weight were reduced in laying quails kept under cyclic thermal stress (7 h at 34 ± 2°C), followed by thermal comfort (17 h at 22 ± 2°C).

The rise in temperatures in all regions of the quail's body and the mean surface and body temperature at the stress temperature are physiological mechanisms used to dissipate excess body heat, such as peripheral vasodilation with blood flow deviation to the surface areas mainly for regions without feathers (Castro et al., 2017), which is an efficient heat dissipation mechanism by sensitive means. Santos et al. (2019) mentioned that temperature influences the surface temperature of Japanese quails, and at high temperatures, these birds exhibit stress behaviors, such as greater displacement for water consumption and ventilated spaces, which can affect their physiological and productive variables (Silva et al., 2017).

However, levels of up to 350 mg $L^{-1}$ of magnesium in water did not affect the percentages of albumin and eggshells, showing no change in the absorption of minerals in the feed. Vohra (1972) mentioned that the magnesium level of 150 mg $kg^{-1}$ in the diet provided good growth in quails, with high mortality at levels of 500 mg $kg^{-1}$. Lima et al. (2020) found no effects of sodium levels (0.08–0.24%) in the diet on the percentage of yolk, albumin, and eggshell of Japanese quails.

The thermal comfort zone in which quail egg production is maximized ranges between 23 and 26°C (El-Tarabany, 2016; Soares et al., 2021a), but even when quails are kept in an environment above this zone. In the thermal comfort zone, egg quality was not influenced by environmental conditions. The quality of quail eggs kept in warm environments was reported by Guimarães et al. (2014) and Vercese et al. (2012).

The temperature of 32°C did not affect egg shell thickness (Ma et al., 2014). Seo et al. (2010) mentioned that supplementation with magnesium oxide in the feeding of laying hens provided better egg thickness, weight, and quality.

Increasing the ambient temperature (20, 24, 28, and 32°C) can reduce the quality of eggshells (Soares et al., 2021a). However, this temperature effect was not externalized, and the average percentage of shell was 8.2%, similar to the values cited by Soares et al. (2021a) when working with laying quail kept at different temperatures (20, 24, 28, and 32°C).

## 5 CONCLUSIONS

The temperature of the head was higher in birds consuming water with levels of magnesium. The percentage of yolk showed a quadratic effect with the increase in magnesium levels, and the temperature of 32°C did not affect the quality of eggs. The magnesium supplementation in the water reduced the effects of thermal stress on the physiological variables and on the quality of the quail eggs, and these birds in the production phase can consume water with magnesium levels of up to 350 mg $L^{-1}$ and be kept at temperatures of up to 32°C for 8 h daily.

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