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Yeast cell wall in the diet of Japanese quails in the laying phase at different stocking densities

Parede celular de levedura em dieta para codornas japonesas na fase de postura em diferentes densidades de criação

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Abstract: The objective of this study was to measure the zootechnical performance and egg quality of Japanese quails housed at different densities and fed diets containing yeast cell walls (YCWs). Five hundred and seventy-six quail (*Coturnix coturnix japonica*) were distributed at 43 weeks of age, and 76% were laid, with an initial weight of 158.50 ± 5.41 g, in a completely randomized design in a 3 × 2 factorial arrangement (three YCW levels: 0, 500, and 750 g.ton^{-1} and two housing densities: 81.5 and 92.4 cm^2/quail), with six replicates of 17 and 15 quail per experimental unit, respectively. The following parameters were evaluated: feed intake, egg production/bird/day, egg production/housed quail, marketable egg production, egg mass, feed conversion per dozen eggs, egg mass and viability, egg weight, specific egg weight, percentage of yolk, albumen and shell, and shell thickness. The means of the three cycles of 21 days were subjected to analysis of variance using the statistical software Sisvar. There was no significant interaction effect between YCW inclusion level and cage density on zootechnical performance parameters or egg quality, except for egg weight, which suggested that YCW addition, regardless of cage density, did not affect the results. It was observed that the eggs of quails housed in cages with 92.4 cm^2/bird feed and 500 g.ton^{-1} YCW had greater egg weights. Shell thickness was independently influenced by cage density, and the lowest density (92.4 cm^2/bird) promoted greater shell thickness. The inclusion of 500 g.ton^{-1} of yeast cell wall material in the diet of Japanese quails housed at a density of 92.4 cm^2/bird improved egg weight and shell thickness without negatively affecting the other parameters of egg quality or zootechnical performance.

Keywords: Quail farming; Shell thickness; Prebiotics; Egg production.

Resumo: Objetivou-se mensurar o desempenho zootécnico e a qualidade de ovos de codornas japonesas alojadas sob diferentes densidades e alimentadas com rações contendo parede celular de levedura (PCL). Foram utilizadas 576 codornas japonesas (*Coturnix japonica*) com 43 semanas de idade e 76% de postura, com peso inicial de 158,50 ± 5,41 g distribuídas em delineamento inteiramente ao acaso em esquema fatorial 3 x 2 (três níveis de PCL: 0; 500 e 750 g.ton^{-1} e duas densidades de

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alojamento: 81,5 e 92,4 cm^2/ave), com seis repetições de 17 e 15 codornas por unidade experimental, respectivamente. Foram avaliados: consumo de ração, produção de ovos/ave/dia, produção de ovos/ave alojada, produção de ovos comercializáveis, massa de ovos, conversão alimentar por dúzia e por massa de ovos e viabilidade das aves; peso do ovo, peso específico, porcentagem de gema, de albúmen e de casca e espessura da casca. Não houve interação entre os níveis de inclusão de PCL e densidade de alojamento para os parâmetros avaliados, exceto para peso do ovo. Codornas alojadas em gaiolas com 92,4 cm^2/ave alimentadas com 500 $g.ton^{-1}$ de PCL apresentaram maior peso do ovo. A espessura de casca foi influenciada de forma independente pela densidade de alojamento, a menor densidade (92,4 cm^2/ave) promoveu maior espessura de casca. A inclusão de 500 $g.ton^{-1}$ de PCL na ração de codornas japonesas alojadas sob densidade de 92,4 cm^2/ave melhora o peso dos ovos e a espessura da casca.

Palavra-chave: Coturnicultura; Espessura da casca; Prebióticos; Produção de ovos.

1. Introduction

In recent years, quail farming has been rapidly developing, emerging as an important productive activity within the national poultry sector (1), achieving high levels of production, a result of technological innovations in the production sector and changes in genetics, nutrition, environment, and health areas(2).

Even in the most technologically advanced breeding facilities, one of the most feared sanitary problems is the emergence of salmonellosis in the flock. This is primarily due to high breeding densities. According to Martins *et al.* [3], in cases where it is possible to identify the etiological agent involved in food poisoning, S. enteritidis is present in approximately 1/3 of these cases. Salmonella enteritidis is one of the most widely distributed serovars worldwide, and asymptomatic birds are associated with chronic infections in adult birds. This can reduce the productive indices of the flock, which can persist in the bird's body for numerous weeks after infection [4].

An important tool used to reduce Salmonella sp. infection in birds is to increase resistance by stimulating the immune system through the use of probiotics, such as beta-glucans and mannan oligosaccharides [5], or through the use of bactericidal products, such as short-chain organic acids, such as fumaric acid, formic acid, and propionic acid, either alone or in combination [6].

Many gram-positive bacteria, such as Lactobacilli and Bifidobacterium, are present in bird excreta, but they are not necessarily related to sanitary problems. However, the frequent presence of pathogens in the breeding environment, as stated by Marmion [7], especially Enterobacteria- and bacteria-causing zoonoses, such as Salmonella and *Escherichia coli*, generally raises concerns due to potential problems in birds and eventually in consumer health.

Furthermore, to combat these pathogens and assist in bird performance, the administration of growth-promoting antibiotics in animals has been increasingly discussed. However, this approach has been discouraged due to concerns about the selection and potential transmission of resistance to these compounds in humans, especially if the

antimicrobial agent registered for use in animals belongs to the same class as the drugs used in human medicine.

According to Ghimpețeanu *et al.* [8], the main aspects that characterize this phenomenon are related to the presence of residual antibiotic growth promoters in animal products and the potential harm they could cause to consumer health. The use of antimicrobial agents in production animals may increase the incidence/prevalence of bacteria that are resistant to these drugs.

The use of additives in bird feed to maintain intestinal health and immune system function is imperative, as the intensification of quail egg production systems has impacted quality of life and raised concerns about animal welfare.

According to Pavan *et al.* [9] along with the intensification of quail egg production systems, there are impacts on the quality of life of the animals and consequent concerns about animal welfare. At present, the cage system is the most commonly used housing system for quail, as it facilitates overall management, reduces labor costs, and allows for increased breeding and production density per area. The use of quail housing density has been studied for reducing egg production costs and maximizing the occupancy of sheds [10,11].

According to El-Tarabany [12], the productive efficiency of quail, as well as the growth and development of their productive apparatuses, are influenced by the housing density used in different stages of breeding. For efficient performance during the laying phase, a density of 107.64 cm2/bird is recommended.

Food additives will not solve issues related to management, health plans, vaccination, nutrition, or water quality, among other factors; however, they can be tools for control and prevention. Intensive animal production is a highly challenging environment, and strengthening the immune system may be one of the key factors for increased productivity [13]. Thus, to improve bird performance in intensified breeding and production systems, additional research is needed to evaluate the use of prebiotics in quail subjected to different housing densities.

The objective of this study was to evaluate the performance and quality of eggs obtained from Japanese quails fed feed containing different amounts of yeast cell walls and housed in battery cages at different densities.

2. Materials and Methods

The experiment was conducted in the Quail Farming Section of the Zootechny Academic Department at the Federal Institute of Education, Science, and Technology of Southeastern Minas Gerais, Campus Rio Pomba, following approval (Protocol No. 02/2020) by the Ethics Committee on Animal Use in Research (CEUA) of the Federal Institute of Southeastern Minas Gerais.

A total of 576 Japanese quails (*Coturnix coturnix japonica*) that were 43 weeks old and had a 76% laying rate and an initial weight of 158.50 ± 5.41 g were used. The plants were randomly distributed in a factorial design of 3 × 2 (three levels of yeast cell wall: 0, 500, and

750 g.ton-1; two housing densities: 81.5 and 92.4 cm2/bird), with six replicates of 17 and 15 quail in each experimental unit, respectively. The experimental period lasted 63 days and was divided into three periods of 21 days each.

The birds were housed in metal battery cages made of 15 mm carbon steel (CHOCMASTER®) from the Isabela model. The cages had five floors and were 36 cm deep, 157 cm high, and 77 cm wide and contained a removable PVC central divider on each floor. Each cage compartment had an area of 1386 cm^2, with a recommended density of 92.4 cm^2/bird and 15 birds/cage. The cages were equipped with nipple-type drinkers with cups, trough-type feeders, and a zinc-coated steel tray for waste collection.

To maintain constant population densities during the experiment, in the case of any deaths, the date of death and the weight of the quail were recorded. One quail was selected for replacement because it originated from the same batch of quail in the experiment, consumed the same feed, had a weight similar to the average weight, and had an egg production level compatible with the experimental unit where death occurred. Water and the same basal feed specifically formulated for laying quail (Table 1) were provided *ad libitum*, according to the nutritional recommendations of Rostagno *et al.* (14).

Table 1 Composition and nutritional values of the basal experimental diet, in natural matter, for laying Japanese quails

Ingredients:	Quantity (kg)
- Whole Corn	58.76
- Soybean Meal	29.00
- Meat and Bone Meal	3.3
- Degummed Soybean Oil	1.00
- Calcitic Limestone	7.08
- Salt	0.31
- Quail Nucleus[1]	0.55
- Total	100.00

Calculated Nutritional Composition:	2.792.904
- Metabolizable Energy (kcal/kg)	19.500
- Crude Protein (%)	2.320
- Crude Fiber (%)	0.931
- Digestible Lysine (%)	0.648
- Digestible Methionine + Cystine (%)	0.680
- Digestible Threonine (%)	0.204
- Digestible Tryptophan (%)	1.220
- Arginine (%)	4.037
- Ether Extract (%)	1.918
- Linoleic Acid (%)	3.450
- Calcium (%)	0.410
- Available Phosphorus (%)	0.160
- Sodium (%)	207.649

[1]*Product Basic Composition: Vitamin A, Vitamin D3, Vitamin E, Vitamin K3, Vitamin B1, Vitamin B2, Calcium Pantothenate, Vitamin B6, Vitamin B12, Niacin, Folic Acid, Biotin, Choline Chloride, Iron Sulfate, Manganese Sulfate, Zinc Sulfate, Calcium Iodate, Sodium Selenite, Copper Sulfate, Cobalt Carbonate, Silicon Dioxide, L-Threonine, DL-Methionine, Enzymatic Additive,* ***Bacillus licheniformis****,* ***Bacillus subtilis****, Antioxidant BHT. Guaranteed levels: vitamin A (min) 2,000,000 IU; vitamin D3 (min) 400,000 IU; vitamin E (min) 7,000 IU; vitamin K3 (min) 400 mg; vitamin B1 (min) 400 mg; vitamin B2 (min) 1,200 mg; pantothenic acid (min) 4,000 mg; vitamin B6 (min) 800 mg; vitamin B12 (min) 2,400 mcg; nicotinic acid (min) 6,000 mg; folic*

acid (min) 200 mg; biotin (min) 30.4 mg; choline (min) 52.8 g; iron (min) 12 g; manganese (min) 16 g; cobalt (min) 60 mg; zinc (min) 12 g; iodine (min) 200 mg; selenium (min) 40 mg; threonine (min) 39.2 g; methionine (min) 240 g; and phytase 240,000 UFT (phytase units).

The yeast cell wall was included in the "on top" form, where the determined quantity for each treatment (500 and 750 g.ton-1) was gradually added to the feed mixer beyond the formula. According to the manufacturer, the cell wall is predominantly composed of β-glucans (1.3-1.6) and MOS, which are insoluble polysaccharides that have prebiotic effects.

The daily management involved collecting and counting the eggs; recording the number of broken, cracked, soft-shelled, and shellless eggs; providing feed; cleaning the egg trays; and reading the maximum and minimum temperatures, dry bulb temperature, wet bulb temperature, and relative humidity (RH).

Temperature and humidity were monitored by Thermo hygrometers, with daily readings taken at 11 a.m. throughout the experimental period. The daily recorded averages for minimum and maximum temperatures and relative humidity during the experimental period were 21.5 ± 1.3°C, 31.8 ± 2.3°C, and 50.3 ± 10.7%, respectively. The thermal comfort range, as obtained by Castro *et al.* [16] for laying Japanese quail, was 22°C to 24°C, with 60% relative humidity.

Artificial lighting was controlled by an automatic timer, allowing the lights in the facility to be turned on and off for a total of 16 hours per day, a common practice in commercial farms. The evaluated zootechnical performance parameters included feed consumption, egg production per bird per day, egg production per housed bird, marketable egg production, egg mass, feed conversion per dozen, and bird viability.

Every 21 days, the leftover feed from each experimental plot was weighed and subtracted from the amount of feed provided at the beginning of the period to obtain the feed consumption (g/bird/day).

Egg production was obtained for each period (21 days) by calculating the total number of eggs produced, including broken, cracked, and abnormal eggs (soft-shelled and shell-less), and expressed as a percentage of the number of live birds for that period (egg production per bird per day = the total number of eggs produced/number of days/number of birds in the experimental plot × 100). The number of birds housed at the beginning of the period was calculated as follows: egg production per housed bird = total number of eggs produced/ number of days/number of birds housed on the first day of the experimental period × 100.

To determine the production of marketable eggs every 21 days, the number of broken, cracked, soft-shelled, and shell-less eggs was subtracted from the total egg production. The marketable egg production was subsequently calculated using the following formula: marketable egg production (%) = number of intact eggs produced/number of days/number of birds in the experimental plot × 100.

All intact eggs produced were weighed during the three penultimate days of every 21-day period (18th, 19th, and 20th days) to obtain the average weight. The average weight of the eggs was multiplied by the egg production per bird per day to obtain the total egg mass (g/bird/day).

The feed conversion per dozen eggs was calculated as the ratio of total feed consumption in kilograms to the dozen eggs produced (kg/dozen), and the feed conversion per egg mass was calculated by dividing the feed consumption in kilograms by the total egg mass (kg/kg).

Bird mortality was monitored daily, and the bird viability rate was obtained at the end of the experimental period. The number of live birds was calculated as the difference in the number of dead birds, and the results are expressed as a percentage.

For egg quality evaluation, the following parameters were analyzed: egg weight (g), specific gravity (g/cm^3), percentage of components (yolk, albumen, and shell), and shell thickness.

On the 18th, 19th, and 20th days of each 21st day, all intact eggs were collected, and 24 eggs from each treatment were randomly selected, with six replicates of four eggs each. The eggs from each replicate and each day were individually weighed on a precision balance (0.001 g) and identified.

Subsequently, the specific gravity was measured by immersing the eggs corresponding to each replicate in saline solutions with densities ranging from 1.055 to 1.095 g/cm^3 at intervals of 0.005 g/cm^3, which were duly calibrated using a hydrometer (OM-5565, Incoterm®), according to the methodology described by Oliveira [15].

The yolk was separated, and its weight was recorded on a precision balance (0.001 g). The weight of the albumen was obtained by subtracting the weight of the yolk plus the weight of the shell from the weight of the egg. The weight of the shell was obtained after washing the shell and drying it in a forced-air circulation oven (60°C) for 24 hours.

The percentages of albumen, yolk, and shell were obtained by dividing the weights of the respective components by the weight of the egg and multiplying the result by 100. The shell thickness was measured using a digital micrometer (DIGIMESS® 0-25 mm) after drying and weighing the shell. Measurements were taken at both poles and at the middle of the egg. The shell thickness for each replicate was determined by the arithmetic mean of the three measurements.

Statistical analysis of the zootechnical performance and egg quality data of quail supplemented with yeast cell wall in the feed was performed with the mean of the three cycles of 21 days. The results were subjected to analysis of variance using Sisvar statistical software. The assumptions of normality of the residues were tested using the Shapiro Wilk test, and the homogeneity of variances was evaluated by utilizing Levene's test.

A model was adopted that included the effects of density (cm^2/bird), the level of yeast cell wall addition, and the interaction between these factors. In the case of a significant interaction, the effect of the additive level was split at each bird density using the Tukey test at the 0.05 probability level. In the absence of a significant interaction, the means of housing densities and the level of additivity were compared by the F test and Tukey test, respectively, both at a 0.05 probability level.

3. Results and Discussion

Supplementation of the yeast cell wall in the feed of laying Japanese quail did not influence (p > 0.05) the zootechnical performance parameters (Table 2). However, it was possible to observe, numerically, that the inclusion of 500 g.ton-1 improved the percentage of egg production per housed bird, the percentage of marketable egg production, and the egg mass (g/bird/day).

Table 2 Performance of Japanese quails in the laying phase housed under two densities and fed diets supplemented with increasing concentrations of yeast cell wall components

Levels of YCW inclusion (g/ton.)	Variables							
	Feed consumption (g/bird/day)	Egg Production Bird-day (%)	Egg Production Bird-housed (%)	Marketable Egg Production (%)	Egg Mass (g/bird/day)	Feed Conversion (kg/dozen)	Feed Conversion (kg/kg)	Viability (%)
0	23.39	76.65	76.03	74.98	7.51	0.368	3.12	96.50
500	23.39	77.71	77.48	76.46	7.61	0.363	3.09	95.75
750	23.04	77.11	76.66	75.50	7.58	0.359	3.05	93.66
Density (cm^2/bird)								
81.5	22.33	74.19	73.71	72.71	7.25	0.363	3.09	95.42
92.4	24.22*	80.12*	79.74*	78.58*	7.89*	0.364	3.08	95.19
P value								
Inclusion levels of YCW	0.530	0.887	0.811	0.791	0.892	0.744	0.679	0.497
Density (D)	0.001	0.002	0.002	0.003	0.001	0.867	0.924	0.906
YCW x D	0.182	0.964	0.939	0.938	0.721	0.601	0.740	0.228
CV (%)	3.72	6.91	7.13	7.07	6.84	7.61	7.13	6.33

** Means differ statistically according to the F test (p<0.05). CV: coefficient of variation.*

Density independently influenced the zootechnical performance parameters, except for feed conversion per mass, per dozen eggs, and bird viability. In general, adopting the housing density recommended by the cage manufacturer, which was 92.4 cm^2/bird (15 birds/cage), improved the zootechnical performance parameters. In other words, quail produced more eggs (g/bird/day) and more eggs per day per housed bird, which are also marketable eggs (Table 2).

Sarica *et al*. [17] reported that egg production, egg mass, viability, and weight decreased in semiheavy laying hens at relatively high cage stocking densities (2000; 1000; 667, and 500 cm^2/bird). The authors reported that hens kept at cage densities of 667 cm^2 or 1000 cm^2 produced the same amount of eggs, while those kept at cage densities of 500 cm^2 decreased egg production, with a delay in reaching 50% of the production age.

Quail stocking density did not interfere with egg quality according to Soares *et al.* [18] but it harmed productive performance. The authors concluded that quails kept at lower densities had higher immunoglobulin Y values (IgY, an antibody present in bird egg yolk), promoting better immune status and well-being.

Similarly, as observed by Lima *et al.* [19], who housed laying Japanese quails under different stocking densities (121.4 cm²/bird; 106.2 cm²/bird; 94.4 cm²/bird; 85 cm²/bird), the density effect on feed consumption, egg weight, conversion per egg mass, and feed conversion per dozen eggs was investigated. They found that the 85 cm²/bird density resulted in lower consumption of feed and reduced egg weight.

However, when laying Japanese quails were housed at densities of 112.2 cm²/bird (10 birds per cage), 102 cm²/bird (11 birds per cage), 93.5 cm²/bird (12 birds per cage), and 86.31 cm²/bird (13 birds per cage), Bourdon (20) did not observe a significant effect on feed consumption, feed conversion per mass or dozen eggs, egg mass, or percentage of egg production per bird/day.

There was no interaction ($p > 0.05$) between the inclusion level of yeast cell walls and cage stocking density for the quail egg physical quality parameters (Table 3), except for egg weight. By breaking down the interaction, it was possible to observe that quail housed at a density of 92.4 cm²/bird and fed 500 g/ton of yeast cell wall had a greater egg weight.

Table 3. Physical quality of Japanese quail eggs in the laying phase housed under two densities and fed diets supplemented with increasing amounts of yeast cell wall

Levels of YCW inclusion (g/ton.)	Variables					
	Egg weight (g)	Specific gravity (g/cm³)	Yolk (%)	Albumen (%)	Shell (%)	Shell thickness (mm)
0	9.81	1.079	29.36	62.46	8.18	0.259
500	9.79	1.076	29.48	62.20	8.09	0.259
750	9.84	1.079	29.38	62.39	8.23	0.259
Density (cm²/bird)						
81.5	9.78	1.076	29.44	62.30	8.11	0.256
92.4	9.84	1.079	29.38	62.40	8.22	0.262*
P value						
Inclusion levels of YCW	0.760	0.243	0.867	0.483	0.241	0.949
Density (D)	0.163	0.121	0.765	0.578	0.110	0.027
YCW x D	0.024	0.565	0.187	0.610	0.885	0.642
CV (%)	1.46	0.52	1.96	0.87	2.47	2.74

** Means differ statistically according to the F test (p<0.05). CV: coefficient of variation.*

The shell thickness was independently influenced by housing density, with a lower density (92.4 cm²/bird – 15 quail per cage) recommended by the cage manufacturer promoting greater shell thickness. This possibly occurred due to the larger space available in the cage compared to the density of 81.5 cm², also allowing access to the feeder and, therefore, proper feed consumption by quails housed at lower density, as evidenced in the results presented in Table 2.

A higher housing density and less space per bird can cause heat stress, especially for adult birds with complete feathering, particularly during the laying phase ([1]). Therefore, birds use regulatory mechanisms, such as increased respiratory frequency, leading to increased CO2 (carbon dioxide) excretion, resulting in a shortage of carbonate ions (CO32−) and

consequently carbonic acid (H2CO3) formation. Carbonic acid is important for the formation of calcium carbonate (CaCO3) in the shell gland, which constitutes 98% of the eggshell. Even when calcium is present, this leads to a deterioration in shell quality.

In contrast to the present research, Soares *et al.* [18] reported no significant differences in shell thickness, specific gravity or eggshell weight for quail housed in cages at different densities (121.43 cm^2/bird; 106.25 cm^2/bird; 94.44 cm^2/bird; and 85.00 cm^2/bird). Similarly, Bourdon (20) evaluated the egg quality of quails kept at densities of 112.20 cm^2/bird, 102.00 cm^2/bird, 93.50 cm^2/bird, and 86.31 cm^2/bird.

The breakdown of the interaction effect of the yeast cell wall inclusion level in the quail diet at each housing density showed that quail housed in cages at a density of 92.4 cm^2 and fed 500 g.ton-1 of yeast cell wall had a greater egg weight (Table 4).

Table 4 Effect of the interaction effect of the yeast cell wall inclusion level in the diet of laying Japanese quail at each housing density on egg weight

Density (cm^2/bird)	Levels of yeast cell wall inclusion (g/ton).		
	0	500	750
	Egg weight (g)		
81.5	9.85 a A	9.67 a B	9.81 a A
92.4	9.76 a A	9.91 a A	9.86 a A

a-b Means followed by different lowercase letters in the rows differ statistically according to the Tukey test (p<0.05). A means followed by different uppercase letters in the columns differ statistically according to the F test (p<0.05).

Consistent with these results, Lima *et al.* [19] and Mahrose *et al.* [20] observed a reduction in egg weight in quail housed at higher density, as quail housed at higher density (81.5 cm^2/bird) produced lighter eggs.

Physiological changes induced by environmental stress, such as the available area for the bird, are associated with increases in plasma corticosterone levels, blood glucose, and the heterophil–lymphocyte ratio. These changes may be accompanied by alterations in the body weight, egg production, and egg weight of the animals [21].

Rahimi *et al.* [22], when turkeys were supplemented with yeast cell wall and MOS, observed histomorphological changes in the villi of bird intestines and improvements in villus length and crypt depth, indicating a functionally active epithelium and a slower rate of epithelial turnover. This treatment led to less discomfort in the mucosa due to a healthier gastrointestinal tract in this group than in the control group, despite bacterial challenges. Therefore, according to the authors, an increase in the surface area could result in better absorption of the available nutrients.

This may explain the improvement in quail egg weight. The yeast cell wall added to the quail diet benefited the intestinal absorption of calcium and other nutrients, coupled with lower stress levels for the birds housed at the recommended density (92.4 cm^2/bird). This is evidenced by increased feed consumption, an increase in the percentage of eggs produced, and an increase in shell thickness.

4. Conclusion

The inclusion of 500 g.ton-1 of the yeast cell wall in the diet of Japanese quails housed at a density of 92.4 cm^2/bird improved egg weight without negatively affecting other egg quality parameters or zootechnical performance.

Conflict of interest declaration

The authors declare that they have no conflicts of interest.

Author Contributions

Conceptualization: M. J. Vieira and M. O. Mendonça. Data Curation: M. J. Vieira and M. O. Mendonça, J. K. Valentim. Investigation: M. J. Vieira, J. S. Amaral, and R. A. Zopelaro. Project Management: M. O. Mendonça. Visualization: M. O. Mendonça, J. K. Valentim, and L.F.T Albino. Supervision: M. O. Mendonça. Writing (Original Draft): M. J. Vieira, J. K. Valentim, and E. D. Silva. Writing (Review and Editing): M. O. Mendonça, L.F.T Albino, and J. K. Valentim.

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