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Impact of different housing densities combined with Environmental enrichment on the production of Japanese quail

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ABSTRACT. This study evaluated the zootechnical performance, egg quality, and behavior of Japanese quails in the laying phase housed in cages of different densities and with or without environmental enrichment. A total of 224 quail were used and distributed into two groups: one with a housing density of 122.7 cm^2 $bird^{-1}$ and one with a housing density of 157.8 cm^2 $bird^{-1}$, each with and without environmental enrichment. The results showed that environmental enrichment did not influence the zootechnical performance parameters of the quail. However, housing density independently affected performance, except for egg mass and bird viability. A lower density (157.8 cm^2 $bird^{-1}$) resulted in greater specific egg weight, albumen percentage, and shell thickness. The interaction between density and environmental enrichment revealed that eggs from quail housed at higher density without enrichment had a greater yolk percentage, while quails housed at lower density without enrichment had a greater shell percentage. The tonic immobility time of the quails was not affected by treatment with different densities or environmental enrichment agents. It can be concluded that a lower housing density, with seven quail per cage (157.8 cm^2 $bird^{-1}$), regardless of environmental enrichment, provides better zootechnical performance and egg quality.

Keywords: behavior; performance; density; enrichment.

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Introduction

In recent years, quail farming has been rapidly developing, emerging as a significant productive activity within the poultry industry segment, achieving high production levels. This is a result of technological innovations in the production sector and changes in the fields of genetics, nutrition, the environment, and health (Silva, Sgavioli, Domingues, & Garcia, 2018).

Simultaneously, as quail egg production systems intensify, animal welfare is impacted, prompting concerns about animal well-being. The cage system is currently the most widely used housing system due to its advantages in sanitary management, reduced labor costs, and ability to accommodate higher stocking densities (Aguiar et al., 2021). However, in this system, as noted by the authors, birds are unable to express natural and social behaviors, which affects their quality of life.

Several countries, including the European Union (EU), have enacted legislation to improve animal welfare, requiring changes in the management and housing systems of laying hens. This includes transitioning from cage housing to systems that allow birds to express their natural behaviors. Similar changes have not yet been observed in the production of Japanese quails (European Union, 1999).

In response to this situation, there are two approaches to address the issue of animal welfare: environmental enrichment, which involves enhancing the facilities to make the environment more suitable for the behavioral needs of animals; or the adoption of breeding systems that promote animal welfare (Barbosa Filho, Silva, Silva, & Coelho, 2006).

Second, Silva, Barbosa Filho, Silva, and Piedade (2006) reported that birds raised in enriched cages were able to express all their natural and comfort behaviors, leading to better well-being conditions. They also observed a preference for nesting in enriched cages (Castilho et al., 2015). In contrast, in conventional systems, even in the absence of suitable conditions, birds attempt to perform their natural behaviors, such as scratching and seeking nests, but the inability to express these behaviors exacerbates stress in intensive cage-based production systems (Alves, Silva, & Piedade, 2007).

The presence of curtains contributes to birds' search for nests, characterized by frequent and quick entry into the supposed nest, as they assess the location before laying eggs (Ferreira, Valentim, Machado, & Oliveira, 2022). With increasing egg production, there is a growing need to utilize natural resources to maintain birds in comfortable environments (Barbosa et al., 2006). For many authors, production and animal welfare are considered paradoxical concerning the quality of life of laying birds, with quail stocking density being studied to reduce egg production costs and maximize facility utilization (Kiani, 2022).

Studies related to quail welfare have shown improvements in productivity and profitability but have also raised controversies regarding the environment (Souza et al., 2020). According to Aguiar et al. (2021), the productive efficiency of quails, as well as their growth and reproductive performance, are influenced by the stocking density used in different stages of rearing. It is recommended to maintain a density of 107.64 cm2 bird-1 during the laying phase to achieve efficient performance.

Other studies have demonstrated that stocking density and the use of environmental enrichment in cages are significantly associated with changes in average egg weight and shell quality (Lukanov & Alexieva, 2012). The benefits of environmental enrichment include a reduction in stereotypical behavior, fear, restlessness, depression, feather pecking, cannibalism, cognitive impairment, and mortality rate, which ultimately contribute to increased production (Gvaryahu et al., 1994; Guinebretière, Huneau-Salaun, Huonnic, & Michel, 2012).

When threatened, quails, like other birds, tend to exhibit a characteristic behavior known as tonic immobility. This behavior occurs when birds are exposed to adverse situations, especially those that induce fear or stress (Franco et al., 2022). Thus, the tonic immobility behavior allows for the assessment of stress in this species based on the induction of immobility and the duration of time the birds remain in this state.

We hypothesize that Japanese quails housed in cages at lower stocking densities and provided with environmental enrichment will exhibit better zootechnical performance, including greater egg quality and a shorter duration of tonic immobility, than quails housed at higher stocking densities without environmental enrichment.

Given these considerations, the objective of this study was to evaluate the zootechnical performance, egg quality, and duration of tonic immobility in Japanese quails housed in cages at different densities, with and without environmental enrichment.

Materials and methods

The experiment was conducted in the Quail Farming section of the Poultry Department at the Academic Department of Animal Science at the Federal Institute of Education, Science, and Technology of Southeastern Minas Gerais, Campus Rio Pomba, with a duration of 63 days, divided into three periods of 21 days each.

A total of 224 Japanese quails (*Coturnix coturnix* japonica) aged 17 weeks were used in the laying phase; the participants had an average weight of 180.02 ± 4.85 grams and a laying percentage of 90.63%. The quails were distributed in accordance with a completely randomized design in a 2 × 2 factorial scheme (two housing densities – 122.7 and 157.8 cm^2/bird; with and without environmental enrichment), with seven replicates of nine and seven quail in each experimental unit, respectively.

In the cages with environmental enrichment, blue TNT (trinitrotoluene) fabric was used on the floor of the cage to simulate ‘nest’ conditions for egg laying. The blue TNT fabric was cut into strips with movable openings and dimensions of 15 × 15 × 15 cm (width × depth × height) inserted in the rear part of the cage.

The birds were housed in carbon steel metal cages with a 15 mm grid and divided into two horizontal rows, with each row containing 14 compartments measuring 47 cm in depth, 23.5 cm in height, and 23.5 cm in width. Each cage compartment had an area of 1,104.5 cm2 and was equipped with a nipple drinker and individual PVC feeders. Excreta were deposited in the pit beneath the cages.

To maintain constant population densities during the research, in the case of any deaths, the date of death and the weight of the deceased quail were recorded. Another bird from the same batch of quail used in the experiment consumed the same feed and had a weight similar to the average weight and egg production level of the experimental group where death occurred; this bird was chosen for replacement.

Water and specific laying quail feed were provided ad libitum, uniformly, to all the experimental units (Table 1). The daily management consisted of collecting and counting the eggs, with daily counts of broken, cracked, soft-shelled, and shellless eggs. Additionally, the feed and cleaned egg collectors and shed corridors were provided.

Table 1. Percentage compositions and nutritional values of the experimental rations in natural matter for laying Japanese quail.

Makings	Quantity (kg)
Corn grain	53.460
Soybean meal	33.500
Soybean oil	2.000
Dicalcium phosphate	1.170
Calcitic limestone	4.870
Quail nucleus in posture[1]	5.000
Mycotoxin adsorbent	0.200
Total	100.000
Calculated nutritional composition	
Energy Metabolizável (kcal kg^{-1})	2.729.954
Crude Protein (%)	19.612
Calcium (%)	3.114
Available Phosphorus (%)	0.452
Sodium (%)	0.172

[1]Basic product composition: vitamin A. vitamin D3. Vitamin E. Vitamin K3. Vitamin B1. Vitamin B2. Calcium pantothenate. Vitamin B6. Vitamin B12. Folic Acid. Biotin. Iron Sulfate. Manganese sulfate. Zinc sulfate. Calcium iodate sodium selenite copper sulfate. Cobalt carbonate. Silicon Dioxide. BHT Antioxidant. Guarantee levels: Vitamin A (min) 1,500,000 I.U. Vitamin D3 (min) 500,000 IU. Vitamin E (min) 1,000 IU. Vitamin K3 (min) 100 mg. The vitamin B1 (min) 30 mg. The vitamin B2 (min) concentration was 1.200 mg. Calcium pantothenate (min) .2.700 mg. The vitamin B6 (min) 300 mg. Vitamin B12 (min) 3,000 mcg. Folic acid (min) 140 mg. Biotin (min) 10 mg. Choline (min) 65 g. Iron (min) 9.000 mg. Manganese (min) 11 g. Cobalt (min) 40 mg. Zinc (min) 12 g. Iodine (min) 150 mg. Selenium (min) 40 mg. . Methionine (min) 200 g.

Artificial lighting was controlled by an automatic timer, allowing for the turning on and off of the shed lights throughout the experimental period, totaling 16 hours of combined natural and artificial photoperiods daily, a procedure commonly used on commercial farms.

The following zootechnical performance parameters were evaluated: feed consumption, egg production per bird per day, production of marketable eggs, egg mass, feed conversion per dozen and egg mass, and bird viability. At the end of each 21-day period, the remaining feed in each experimental plot was weighed and deducted from the initial feed quantity provided to determine daily feed consumption (g bird-1 day-1).

Egg production per bird per day was calculated by dividing the total number of eggs produced, including broken, cracked, abnormal, soft-shelled, and shellless eggs, by the days in the period and the number of birds in the experimental plot. This value was multiplied by 100 and expressed as a percentage (egg production per bird per day = total number of eggs produced/number of days/number of birds in the experimental plot × 100).

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

All intact eggs produced were individually weighed during the last three days of each 21 days (18th, 19th, and 20th days) to obtain the average egg weight. The average egg weight was multiplied by the egg production per bird per day to obtain the total egg mass (g bird-1 day-1).

The feed conversion per dozen eggs was calculated by dividing the total feed consumption in kilograms by the number of dozen eggs produced (kg dozen-1), and the feed conversion per egg mass was calculated by dividing the feed consumption in kilograms by the total egg mass (kg kg-1).

Bird mortality was monitored daily, and at the end of the experimental period, the bird viability rate was calculated by subtracting the number of dead birds from the number of live birds, with the result expressed as a percentage.

To assess egg quality, the following parameters were analyzed: egg weight (g), specific gravity (g cm-3), and percentage of egg components (yolk, albumen, and shell). On the 18th, 19th, and 20th days of each 21st day, all intact eggs were collected, and four eggs from each experimental plot were randomly selected, with seven replicates of six eggs each. The eggs from each replicate and each day were individually weighed on a scale with a precision of 0.001 g and identified.

Subsequently, the specific gravity was measured by immersing the eggs corresponding to each replication in saline solutions with densities ranging from 1.055 to 1.095 g cm^{-3} in intervals of 0.005 g cm^{-3}; the values were properly calibrated using a hydrometer (OM-5565, Incoterm®) following the methodology described by Freitas et al. (2011).

Afterward, the eggs from each replicate and each day were broken in a Petri dish. The yolk was separated, and its weight was recorded on a scale with a precision of 0.001 g. The weight of the albumen was obtained as the difference between the egg weight and the yolk weight plus the shell weight, with the latter obtained after washing and subsequently drying in a forced air circulation oven (60°C) for 24 hours. The percentages of albumen, yolk, and shell were calculated by dividing the weights of the respective components by the egg weight and multiplying the result by 100.

The analyses of tonic immobility time were conducted based on data collected over four days, with the first on the second day of the experiment and the others on the last day before the end of each 21 days. The seven replicates (cages) of each treatment were analyzed two times of day, at 9 am and 3 pm.

For the evaluation of tonic immobility time (TIT), all quails from an experimental unit were placed in a box, and one bird was turned at a time and placed in a dorsal recumbent position on a table. Before the hand was removed from the bird, slight pressure was applied to the animal for three seconds. After this procedure, the time the bird remained immobile was counted using a digital stopwatch. Immobility for at least ten seconds was considered to indicate a state of tonic immobility (Jones & Faure, 1981).

Statistical analyses were performed using the mean values of the three 21-day cycles. The results were subjected to analysis of variance using the Sisvar program. A model was adopted, including the effects of density (cm2 bird) on environmental enrichment and the interactions between these factors. In the case of a significant interaction, the effect of environmental enrichment was analyzed for each bird density using the F test, for which the probability was 0.05.

In the absence of a significant interaction, the means of the use or absence of environmental enrichment and the housing densities were compared using the F test, for which the probability was 0.05. The tonic immobility test was analyzed using the F test with a probability of 0.05, and the results are expressed as the mean time in seconds that the quails remained immobile.

Results and discussion

The daily average temperatures, both minimum and maximum, and the relative humidity recorded during the experimental period were 18.5 ± 2.5°C, 25.3 ± 4.1°C, and 52.5 ± 17.0%, respectively. The thermal comfort range, as determined by Jones & Waddington (1992) for laying Japanese quail, is 22 to 24°C, and the relative humidity is 60%. Based on this information and considering the recorded values for minimum and maximum temperatures, it can be inferred that during part of the experimental period, the quails were exposed to cold stress conditions.

However, these adverse weather conditions did not influence the zootechnical performance of the birds in the present study. Environmental enrichment in the cages of laying Japanese quail did not influence ($p > 0.05$) the zootechnical performance parameters (Table 2), suggesting that the use of environmental enrichment, regardless of housing density, did not negatively affect the results.

Density independently influenced the zootechnical performance parameters, except for egg mass (g bird^{-1} day^{-1}) and bird viability. In general, adopting a lower housing density, i.e., 157.8 cm^2 bird^{-1} (7 birds/cage), improved the zootechnical performance parameters. The quail produced a greater daily egg quantity and a greater percentage of marketable eggs with better feed conversion, both per mass and per dozen eggs (Table 2).

Savory and Lariviere (2000) reported that egg production and egg mass, bird viability, and egg weight decreased in semi heavy laying hens at relatively high cage housing densities (2000; 1000; 667; and 500 cm2 bird-1). The authors reported that hens kept at cage densities of 667 or 1000 cm2 produced the same number of eggs, while those kept in 500 cm2 space decreased egg production with a delay in reaching 50% production age.

Table 2. Performance of Japanese quail housed at two densities in cages with and without environmental enrichment.

Density (cm^2 bird^{-1})	Variables						
	FC (g ve^{-1} day^{-1})	PE Bird-day (%)	PME (%)	EM (g bird^{-1} day^{-1})	AC (kg dozen^{-1})	FC (kg kg^{-1})	Viability (%)
157.8 (7 birds cage^{-1})	27.46*	92.70*	92.26*	9.91	0.356*	2.78*	97.96
122.7 (9 birds cage^{-1})	26.42	82.89	82.64	8.80	0.384	3.01	100.00
Environmental enrichment							
With	26.88	86.84	86.64	9.28	0.373	2.91	86.84
Without	26.99	88.75	88.269	9.42	0.367	2.88	88.75
P value							
Density (D)	0.0195	0.00001	0.00001	0.00001	0.0092	0.0040	0.1701
Enrichment (E)	0.7840	0.2424	0.3251	0.4518	0.5419	0.7259	0.9975
D x E	0.7739	0.4748	0.4765	0.7926	0.5609	0.7444	0.9975
Coefficient of Variation (%)	4.09	4.82	4.88	5.15	7.02	6.79	3.86

*Means differ significantly according to the F test ($p < 0.05$). FC: Feed consumption; PE: bird-day (%): Production of eggs bird^{-1} day^{-1}. PME (%): Production of marketable eggs. EM (g bird^{-1} day^{-1}); egg mass. FC: Feed conversion.

The present research demonstrated a 9.8% increase in egg production per bird per day and a 9.6% increase in the production of marketable eggs for quail housed at lower densities (7 birds cage^{-1}), regardless of the use of environmental enrichment (Table 2).

Similarly, Leandro et al. (2005) observed that housing laying Japanese quail at different housing densities (121.4, 106.2, 94.4, and 85 cm^2 bird^{-1}) affected feed consumption, egg weight, conversion per egg mass, and feed conversion per dozen eggs, and they found that a density of 85 cm^2 bird^{-1} led to lower consumption and reduced egg weight.

However, when Japanese quails were housed in the laying phase at densities of 112.2 (10 birds per cage), 102 (11 birds per cage), 93.5 (12 birds per cage), and 86.31 cm^2 bird^{-1} (13 birds per cage), Santos Bourdon et al. (2021) did not observe a significant effect on feed consumption, feed conversion per mass or per week, egg mass, or the percentage of egg production per bird per day. According to the author, this may have occurred because the animals adapted to the dimensional conditions of the cage.

In contrast to the present research, where a significant effect ($p < 0.05$) was observed for feed conversion per mass and per dozen eggs (Table 2), quail housed at a lower density, 7 birds/cage (157.8 cm^2 bird^{-1}), were more efficient at converting feed into eggs.

There was no significant interaction ($p > 0.05$) between the use of environmental enrichment in the cage and housing density for the physical egg quality parameters of the quail (Table 3), except for egg yolk and eggshell percentage.

Through the breakdown of the interaction, it was observed that quails housed at a density of 157.8 cm^2 bird^{-1} (7 quails per cage) without the inclusion of environmental enrichment had a greater percentage of eggshells, with no change in the yolk percentage (Table 4).

The specific weight (g cm^{-3}), albumen percentage, and shell thickness were independently influenced by housing density, with lower density (157.8 cm^2 bird^{-1} – 7 quail per cage) promoting ($p < 0.05$) higher values for all three parameters. This difference was likely due to the greater space available in the cage, greater access to the feeder, and thus adequate feed consumption by the quails housed at lower density than by those housed at a density of 122.7 cm^2 bird^{-1}, as evidenced in the results presented in Table 3.

Table 3. Physical quality of Japanese quail eggs housed at two densities in cages with and without environmental enrichment.

Density (cm^2 bird^{-1})	Variables					
	Egg weight (g)	Specific gravity (g cm^{-3})	Egg yolk (%)	Albumen (%)	Eggshell (%)	Shellness (mm)
157.8 (7 birds cage^{-1})	10.69	1.076*	26.96	65.43*	7.61	0.256*
122.7 (9 birds cage^{-1})	10.61	1.074	27.30	64.99	7.71	0.248
Environmental enrichment in the cage						
With	10.69	1.075	26.90	65.59*	7.51	0.252
Without	10.61	1.075	27.36*	64.83	7.81*	0.252
P value						
Density (D)	0.3836	0.0035	0.0591	0.0242	0.1024	0.0025
Enrichment (E)	0.3672	0.3336	0.0134	0.0003	0.0000	0.9209
D x E	0.3217	0.1341	0.0022	0.0831	0.0001	0.5308
Coefficient of Variation (%)	2.03	0.12	1.66	0.73	1.96	2.24

*Means differ statistically according to the F test ($p < 0.05$).

Table 4. The interaction effect of quail housing density and environmental enrichment in the cage on yolk and eggshell percentage was evaluated.

Environmental enrichment	Density (cm^2 bird^{-1})	
	157.8 (7 birds cage^{-1})	122.7 (9 birds cage^{-1})
Egg yolk (%)		
With	27.03 A a	26.78 B a
Without	26.90 A b	27.82 A a
Eggshell (%)		
With	7.33 B b	7.69 A a
Without	7.90 A a	7.73 A a

a-b Means followed by distinct lowercase letters in the lines differ statistically according to the F test ($p < 0.05$). A-B Means followed by distinct capital letters in the columns differ statistically according to the F test ($p < 0.05$).

The eggshell is composed of calcium carbonate (CaCO3) and is formed in the quail oviduct (Diana et al., 2021). In this location, calcium, which is absorbed from the diet, combines with carbonic acid (HCO3), which is derived from the CO2 produced during the respiratory process and reacts with water through the action of the enzyme carbonic anhydrase (Gautron et al., 2021).

Respiratory disturbances caused by thermal stress, along with density factors where there are more birds, potentially hindering feed intake, can directly affect shell formation. This was also observed in the present study, where a lower density resulted in thicker eggshells (Table 3).

However, contrary to these findings, Pavan, Garcia, Móri, Pizzolante, and Piccinin (2005) did not find significant differences in eggshell thickness for quail housed in cages at different densities (121.43; 106.25; 94.44; and 85 cm^2 $bird^{-1}$). Similarly, Santos Bourdon et al. (2021) evaluated the egg quality of quail kept at 112.20, 102.00, 93.50, and 86.31 cm^2 $bird^{-1}$ and did not find significant differences in eggshell thickness.

The breakdown of the interaction effect of the inclusion level of environmental enrichment in quail cages at each housing density showed that eggs from quail housed at a density of 122.7 cm^2 $bird^{-1}$ without enrichment had a higher yolk percentage, and quails housed at a lower density (157.8 cm^2 $bird^{-1}$) without enrichment had a higher eggshell percentage (Table 4).

Lopes, Fuentes, Freitas, Soares, and Ribeiro (2006) did not observe significant effects of housing density, dietary metabolizable energy levels, or their interaction on egg weight, yolk percentage, or albumen percentage. However, they found that energy levels influenced specific gravity and eggshell percentage, which differs from the findings of the present study, where quails housed at lower densities (7 birds $cage^{-1}$) with environmental enrichment in the cages produced a greater yolk percentage.

Garcia, Murakami, Galli, Oliveira, and Martins (2000) and Lopes et al. (2006) did not detect a significant influence of the number of quail per cage on eggshell percentage. Garcia et al. (2000) reported a linear increase in eggshell percentage with increasing dietary energy levels but did not detect an influence of the number of quail per cage on eggshell percentage.

Physiological changes caused by environmental stress, such as the available area per bird, are associated with increases in plasma corticosterone levels, blood glucose, and the heterophil/lymphocyte ratio. These changes may be accompanied by alterations in body weight, egg production, and egg weight (Botool et al., 2023).

This could explain the improvements in shell thickness (Table 3) and the percentages of yolk and eggshell material in the quail eggs (Table 4), as well as the lower stress levels in birds housed at lower density (157.8 cm^2 $bird^{-1}$), which was demonstrated by increased feed consumption and an increase in the percentage of eggs produced and, consequently, in the percentage of marketable eggs (Table 2).

The duration of tonic immobility in Japanese quails housed in cages at two densities, with or without environmental enrichment, was not altered ($p > 0.05$). A lower housing density and/or environmental enrichment did not significantly change the stress level of the birds (Table 5). Tonic immobility was considered when the bird remained immobile for at least ten seconds (Jones & Faure, 1981).

According to Jones and Faure (1981), one way to quantify the level of fear is through the evaluation of tonic immobility (TI) duration, characterized by a reduced response to external stimuli. Therefore, the duration of TI indicates the level of fear; in other words, the longer an animal remains in a catatonic state, the more frightened it is. It can be inferred from this study that environmental enrichment in the cage numerically reduced fear intensity, as the quails spent 37 s less immobile, indicating a quicker recovery (Table 5).

Table 5. Tonic immobility time of Japanese quail housed under two densities in cages with and without environmental enrichment.

Density (cm^2 $bird^{-1}$)	Time in Tonic Immobility (s)*
157.8 (7 birds $cage^{-1}$)	6.38
122.7 (9 birds $cage^{-1}$)	5.93
Environmental enrichment	
With	5.97
Without	6.34
P value	
Density (D)	0.3615
Enrichment (E)	0.4551
D x E	0.7079
Coefficient of Variation (%)	20.66

*Not significant ($p > 0.05$).

The tonic immobility test is considered a natural behavior related to stress; that is, the more stressed a bird is, the longer the duration of this state (Savory and Lariviere, 2000). Tonic immobility belongs to the category of defensive behavior and is initially preceded by coping behavior and responses evoked by a stressful situation (Marques et al., 2010). According to Michelan, Michelan, Paula, and Hoshino (2006), this behavior is the last response to anti-predatory defense in some species and is characterized by pretending to be dead to gain an opportunity to escape by inducing relaxation of the predator's attention.

Marques et al. (2010) fed quails a control diet and diets containing 250, 500, or 750 mg of Matricaria chamomilla kg-1 of feed and reported that the tested chamomile levels did not significantly affect the duration of tonic immobility in quails during the laying phase. These results are similar to those obtained by Gravena et al. (2009), who did not observe significant differences between treatments in the duration of tonic immobility in laying quails when different concentrations of Valeriana officinalis, another herbal remedy with anxiolytic properties, were added to the feed.

Different results were found by Marques et al. (2010) when quail diets were supplemented with increasing levels of *Passiflora alata*, another herbal remedy with calming properties. The authors observed that birds fed feed without the additive had a longer duration of tonic immobility than did those receiving Passiflora.

The results of the tonic immobility duration showed that the coefficient of variation was high, which can be explained by the individual variation in each quail (Table 5), as also highlighted by Marques et al. (2010).

In general, independent of housing density, a lower density of 7 quail cage-1 (158.7 cm-2 bird-1) improved ($p < 0.05$) the following zootechnical performance parameters: feed consumption, egg production per bird-day, production of marketable eggs, feed conversion per dozen eggs, and feed conversion per mass. A lower density also led to an improvement ($p < 0.05$) in egg physical quality parameters, namely, specific gravity, albumen percentage, and shell thickness.

Numerically and independently, both the lower density of the 7 quail/cage (158.7 cm-2 bird-1) and the inclusion of environmental enrichment in the cages reduced the duration of tonic immobility (TIT), indicating a potential predisposition to reduced stress.

Conclusion

A lower stocking density, with seven quail per cage (158.7 cm^{-2} $bird^{-1}$), regardless of the use of environmental enrichment, results in better zootechnical performance and egg quality; however, it does not affect the tonic immobility time of quails in the laying phase.

References

Aguiar, D. P., Valentim, J. K., Lima, H. J. D., Bittencourt, T. M., Andreoti, L. Z., Pereira, I. D. B., ... Zanella, J. (2021). Beak trimming and stocking densities for laying and performance traits and behavioral patterns in Japanese quails. *Revista de Investigaciones Veterinarias del Perú, 32*(5), e19248. DOI: https://doi.org/10.15381/rivep.v32i5.19248

Alves, S. P., Silva, I. J. O., & Piedade, S. M. S. (2007). Avaliação do bem-estar de aves poedeiras comerciais: efeitos do sistema de criação e do ambiente bioclimático sobre o desempenho das aves e a qualidade de ovos. *Revista Brasileira de Zootecnia, 36*(5), 1388-1394. DOI: https://doi.org/10.1590/S1516-35982007000600023

Barbosa Filho, J. A. D., Silva, M. A. N., Silva, I. J. O., & Coelho, A. A. D. (2006). Egg quality in layers housed in different production systems and submitted to two environmental conditions. *Revista Brasileira de Ciência Avícola, 8*(1), 23-28. DOI: https://doi.org/10.1590/S1516-635X2006000100003

Batool, F., Bilal, R. M., Hassan, F. U., Nasir, T. A., Rafeeque, M., Elnesr, S. S., ... & Alagawany, M. (2023). An updated review on behavior of domestic quail with reference to the negative effect of heat stress. Animal Biotechnology, 34(2), 424-437. DOI: 10.1080/10495398.2021.1951281

Castilho, V. A. R., Garcia, R. G., Lima, N. D. S., Nunes, K. C., Caldara, F. R., Nääs, I. A., ... Jacob, F. G. (2015). Welfare of laying hens in different densities of housing. *Revista Brasileira de Engenharia de Biossistemas, 9*(2), 122-131. DOI: https://doi.org/10.18011/bioeng2015v9n2p122-131

Diana, T. F., Calderano, A. A., Tavernari, F. C., Rostagno, H. S., Teixeira, A. O., & Albino, L. F. T. (2021). Age and calcium sources in laying hen feed affect calcium digestibility. *Open Journal of Animal Sciences, 11*(3), 501-513. DOI: https://doi.org/10.4236/ojas.2021.113034

European Union. (1999). *Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens*. Luxemburgo: Official Journal of the European Communities.

Ferreira, J. A., Valentim, J. K., Machado, L. C., & Oliveira, H. F. (2022). Aplicação de etogramas no bem-estar de aves: uma revisão de literatura. *Uniciências*, *26*(1), 24-30. DOI: https://doi.org/10.17921/1415-5141.2022v26n1p24-30

Franco, B. R., Shynkaruk, T., Crowe, T., Fancher, B., French, N., Gillingham, S., & Schwean-Lardner, K. (2022). Light color and the commercial broiler: effect on behavior, fear, and stress. *Poultry Science, 101*(11), 102052. DOI: https://doi.org/10.1016/j.psj.2022.102052

Freitas, L. W., Paz, I. C. L. A., Garcia, R. G., Caldara, F. R., Seno, L. O., Felix, G. A., ... Cavichiolo, F. (2011). Aspectos qualitativos de ovos comerciais submetidos a diferentes condições de armazenamento. *Agrarian, 4*(11), 66-72.

Garcia, E. R. M., Murakami, A. E., Galli, J. R., Oliveira, C. A. L., & Martins, E. N. (2000). Efeito do nível energético e da densidade populacional sobre o desempenho de codornas (*Coturnix coturnix* japonica) em postura. *Revista Brasileira de Ciência Avícola, 2*(supl. 2), 47.

Gautron, J., Stapane, L., Le Roy, N., Nys, Y., Rodriguez-Navarro, A. B., & Hincke, M. T. (2021). Avian eggshell biomineralization: an update on its structure, mineralogy and protein tool kit. *BMC Molecular and Cell Biology, 22*(11), 1-17. DOI: https://doi.org/10.1186/s12860-021-00350-0

Gravena, R. A., Marques, R. H., Silva, J. D. T., Hada, F. H., Silva, V. K., Munari, D. P., & Moraes, V. M. B. (2009). Uso da Valeriana officinalis em dietas de codornas japonesas na fase de postura. *Biotemas, 22*(4), 185-191. DOI: https://doi.org/10.5007/2175-7925.2009v22n4p185

Guinebretière, M., Huneau-Salaun, A., Huonnic, D., & Michel, V. (2012). Cage hygiene, laying location, and egg quality: the effects of linings and litter provision in furnished cages for laying hens. *Poultry Science, 91*(4), 808-816. DOI: https://doi.org/10.3382/ps.2011-01881

Gvaryahu, G., Ararat, E., Asaf, E., Lev, M., Weller, J. I., Robinzon, B., & Snapir, N. (1994). An enrichment object that reduces aggressiveness and mortality in caged laying hens. *Physiology & Behavior*, *55*(2), 313-316. DOI: https://doi.org/10.1016/0031-9384(94)90139-2

Jones, R. B., & Faure, J. M. (1981). Sex and strain comparisons of tonic immobility ("righting time") in the domestic fowl and the effects of various methods of induction. *Behavioral Processes, 6*(1), 47-55. DOI: https://doi.org/10.1016/0376-6357(81)90015-2

Jones, R. B., & Waddington, D. (1992). Modification of fear in domestic chicks, Gallus gallus domesticus, via regular handling and early environmental enrichment. *Animal Behavior, 43*(6), 1021-1033. DOI: https://doi.org/10.1016/S0003-3472(06)80015-1

Kiani, A. (2022). Effects of group sizing on behavior, welfare, and productivity of poultry. *Journal of World's Poultry Research, 12*(1), 52-68. DOI: https://doi.org/10.36380/jwpr.2022.7

Leandro, N. S. M., Vieira, N. S., Matos, M. S., Café, M. B., Stringhini, J. H., & Santos, D. A. (2005). Desempenho produtivo de codornas japonesas (*Coturnix coturnix* japonica) submetidas a diferentes densidades e tipos de debicagem. *Acta Scientiarum. Animal Sciences, 27*(1), 129-135. DOI: https://doi.org/10.4025/actascianimsci.v27i1.1263

Lopes, I. R. V., Fuentes, M. F. F., Freitas, E. R., Soares, M. B., & Ribeiro, P. S. (2006). Efeito da densidade de alojamento e do nível de energia metabolizável da ração sobre o desempenho zootécnico e características dos ovos de codornas japonesas. *Revista Ciência Agronômica, 37*(3), 369-375.

Lukanov, H., & Alexieva, D. (2012). Performance of three commercial hybrid layers housed in conventional and enriched cage systems. *Journal of Agricultural Science and Technology, 4*(3), 246-249.

Marques, R. H., Gravena, R. A., Silva, J. D. T., Hada, F. H., Silva, V. K., Munari, D. P., & Moraes, V. M. B. (2010). Camomila como aditivo fitoterápico para codornas na fase de postura. *Revista Brasileira de Saúde e Produção Animal, 11*(4), 990-998.

Michelan, C. M., Michelan, L. D., Paula, H. M. G., & Hoshino, K. (2006). Imobilidade tônica e imobilidade do nado forçado em cobaias. *Revista de Etologia, 8*(2), 89-95.

Pavan, A. C., Garcia, E. A., Móri, C., Pizzolante, C. C., & Piccinin, A. E. (2005). Efeito da densidade na gaiola sobre o desempenho de poedeiras comerciais nas fases de cria, recria e produção. *Revista Brasileira de Zootecnia, 34*(4), 1320-1328. DOI: https://doi.org/10.1590/S1516-35982005000400029

Santos Bourdon, V. D. D., Souza, R. G., Nascimento Oliveira, E. J., Vieira, D. V. G., Moron, S. E., Vaz, R. M. G. V., ... Costa, F. G. P. (2021). Productive performance, thermal and blood parameters of Japanese laying quails at different cage stocking densities. *Research, Society and Development*, *10*(3), DOI: https://doi.org/10.33448/rsd-v10i3.13686

Savory, C. J., & Lariviere, J. M. (2000). Effects of qualitative and quantitative food restriction treatments on feeding motivational state and general activity level of growing broiler breeders. *Applied Animal Behavior Science*, 69(2), 135-147. DOI: https://doi.org/10.1016/S0168-1591(00)00123-4

Silva, A. F., Sgavioli, S., Domingues, C. H. F., & Garcia, R. G. (2018). Coturnicultura como alternativa para aumento de renda do pequeno produtor. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 70*(3), 913-920. DOI: https://doi.org/10.1590/1678-4162-10065

Silva, I. J. O., Barbosa Filho, J. A. D., Silva, M. A. N., & Piedade, S. M. S. (2006). Influência do sistema de criação nos parâmetros comportamentais de duas linhagens de poedeiras submetidas a duas condições ambientais. *Revista Brasileira de Zootecnia, 35*(4), 1439-1446. DOI: https://doi.org/10.1590/S1516-35982006000500025

Souza, A. V., Morais, M. V. M., Rocha, M. C., Souza, R. M., Valentim, J. K., Pietramale, R. T. R., ... Lima, H. J. D. (2020). Influence of fennel in Japanese Quail Diet over egg quality and behavior aspects. *Boletim de Indústria Animal, 77*, 1-13.