







# Impact of herbal medicines use on the welfare of laying Japanese quails

*Impacto do uso de ervas fitoterápicas no bem-estar de codornas japonesas em postura*

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**Received:** 2022 Apr 16 | **Accepted:** 2023 Mar 8

DOI: <http://dx.doi.org/10.7213/acad.2023.21001>

Rev. Acad. Ciênc. Anim. 2023;21:e21001

the behavior of laying Japanese quails, however, they were able to decrease the tonic immobility time of the birds, with chamomile being the herb that exercised greater effect.

**Keywords:** Animal welfare. Behavior. Stress. Tonic immobility.

## Resumo

*Objetivou-se com esse estudo avaliar o efeito da camomila, erva-cidreira e valeriana na modulação do estresse, avaliando o comportamento e imobilidade tônica de codornas japonesas poedeiras. O delineamento experimental foi inteiramente casualizado com quatro dietas e seis repetições contendo quatro codornas poedeiras (*Coturnix japonica*) por unidade experimental, totalizando 96 aves. Os fitoterápicos estudados foram tratamento controle; 500 mg de camomila (*Matricaria recutita* L.); 500 mg de erva-cidreira (*Melissa officinalis* L.); 500 mg de valeriana por kg de ração (*Valeriana officinalis* L.). Não houve diferença significativa ( $p < 0,05$ ) na frequência dos comportamentos das aves. No entanto a imobilidade tônica apresentou diferença ( $p > 0,05$ ) entre os tratamentos. O uso das ervas camomila, erva-cidreira e valeriana (500 mg/kg de ração) não influenciou o comportamento de codornas japonesas*

## Abstract

The objective of this study was to evaluate the effect of chamomile, lemon balm, and valerian on stress modulation, evaluating the behavior and tonic immobility of laying Japanese quail. The experimental design was completely randomized with four diets and six replications containing four laying quails (*Coturnix japonica*) per experimental unit, totaling 96 birds. The herbal medicines studied were control treatment; 500 mg of chamomile (*Matricaria recutita* L.); 500 mg of lemon balm (*Melissa officinalis* L.); 500 mg of valerian per kg feed (*Valeriana officinalis* L.). There was no significant difference ( $p < 0.05$ ) on the frequency of bird behaviors. However, tonic immobility showed a difference ( $p > 0.05$ ) between treatments. The use of chamomile, lemon balm, and valerian herbs (500 mg/kg feed) did not influence

em postura, porém, foram capazes de diminuir o tempo de imobilidade tônica das aves, sendo a camomila a erva que exerceu maior efeito.

**Palavras-chaves:** Bem-estar animal. Comportamento. Estresse. Imobilidade tônica

## Introduction

Coturniculture has been growing and gaining space annually in Brazilian agriculture, both in the production of eggs and in the production of meat. Due to the high demand for food, coupled with the low investment cost, and rapid productive return, studies related to genetic improvement, nutrition, management, health and technification, have made the activity more and more professionalized and intensified.

To intensify the production systems, management such as densification of birds in conventional cages were adopted in the sense; however, the constant competition for space and food has caused a negative impact on the well-being of birds (Pavan et al., 2005).

Quails, when stressed, decrease the production of eggs, increase the expression of aggressive and agitated behaviors, resulting in a greater number of injuries caused by the pecking of feathers markedly, also increasing the energy waste directed to the expression of these behavioral responses (Silva et al., 2010b). The injuries caused by aggressive pecking are an extremely important problem, as it concerns the welfare of quails (Wechsler e Schmid, 1998).

Currently, animal welfare is gaining prominence in animal production, as this concern is interesting for the breeding system. In this context, consumers of products of animal origin have shown themselves to be in favor of adopting measures that favor this aspect. Thus, measures to control stress in animals become important for the success of breeding, reflecting positively on the production and welfare of birds.

Through the study of medicinal plants and their applications in the prevention and/or cure of diseases, phytotherapy has been explored to try to minimize the stress of birds. Some plants such as chamomile, green tea, and lemongrass have the property of calming and minimizing stress in humans (Rocha et al., 2008).

These effects have also been proven in research with birds (Sarker et al., 2010; Lourenço et al., 2013; Royer et al., 2015). Some plants have already been evaluated to minimize stress in Japanese quails (Abaza et al., 2003; Marques et al., 2010; Galib et al., 2011; Mahmmoud, 2013). However, most studies reported in the literature have analyzed the effect of herbal medicine on performance and blood parameters and little is known about the expression of behaviors under the influence of herbs, making it necessary to study behavioral responses, since it is closely linked to well-being.

The objective of this study was to evaluate the effect of chamomile, lemon balm, and valerian on stress modulation, evaluating the behavior and tonic immobility of laying Japanese quail.

## Material and methods

The experiment was carried out in the coturniculture sector of the Experimental Farm of the Universidade Federal de Mato Grosso, located in the municipality of Santo Antônio de Leverger, MT, Brazil. The project was submitted, analyzed, and approved by the ethics committee on the use of animals (CEUA) under number 23108.066706/2020-93.

A total of 96 laying quails (*Coturnix japonica*) were used, weighing  $173.9 \pm 0.004$  g, at 48 weeks of age, with an initial laying rate of  $72.48 \pm 3.54\%$ . A completely randomized design with four diets was used: control treatment; 500 mg of chamomile (*Matricaria recutita* L.); 500 mg of lemon balm (*Melissa officinalis* L.); 500 mg of valerian per kg feed (*Valeriana officinalis* L.); and six replicates, containing four birds per experimental unit. The experiment lasted 42 days, being divided into two periods of 21 days each.

The experimental diets (Table 1) were formulated based on corn and soybean meal, being isoenergetic and isoproteic, according to the recommendations and nutritional compositions of Rostagno et al. (2017) for laying Japanese quails. The herbs used in the feed were bought dry and then ground to a fine powder.

The birds were housed in 30 galvanized wire cages, horizontally arranged, with the dimensions of 50 x 38 x 21 cm (length x width x height), provided with trough type and nipple type water trough. The rations were provided twice a day (8 am and 4 pm) and water at will.

**Table 1** - Ingredient and calculated nutrient content of the diet of laying Japanese quails feed with different herbal medicine in the diet

Ingredients (%)	Medicinal herbs			
	Control	Chamomile	Lemon Balm	Valerian
Corn	54.17	54.12	54.12	54.12
Soybean meal (45%)	34.70	34.70	34.70	34.70
Limestone	7.01	7.01	7.01	7.01
Dicalcium phosphate	1.15	1.15	1.15	1.15
Salt	0.36	0.36	0.36	0.36
Vitamin and mineral supplement <sup>1</sup>	1.50	1.50	1.50	1.50
Soybean oil	1.11	1.11	1.11	1.11
Herb	0.00	0.05	0.05	0.05
<b>Calculated nutritional composition</b>				
Metabolizable energy (kcal/kg)	2800	2800	2800	2800
Crude protein (%)	19.46	19.46	19.46	19.46
Crude fiber (%)	2.74	2.74	2.74	2.74
Calcium (%)	3.07	3.07	3.07	3.07
Available phosphorus (%)	0.30	0.30	0.30	0.30
Sodium (%)	0.16	0.16	0.16	0.16
<b>Digestible amino acids (%)</b>				
Digestible lysine	1.08	1.08	1.08	1.08
Digestible methionine+ Cystine	0.94	0.94	0.94	0.94
Digestible tryptophan	0.23	0.23	0.23	0.23
Digestible threonine	0.68	0.68	0.68	0.68

Note: <sup>1</sup>Vitamin-mineral supplement composition: Calcium (min) 80 g/kg, Calcium (max) 100 g/kg, Phosphorus (min) 37 g/kg, Sodium (min) 20 g/kg, Methionine (min) 21.5 g/kg, Lysine (min) 18 g/kg, Vitamin A (min) 125000 UI/kg, Vitamin D3 (min) 25000 UI/kg, Vitamin E (min) 312 UI/kg, Vitamin K3 (min) 20 mg/kg, Vitamin B1 (min) 20 mg/kg, Vitamin B2 (min) 62.5 mg/kg, Vitamin B6 (min) 37.5 mg/kg, Vitamin B12 (min) 200 mg/kg, Folic Acid (min) 6,25 mg/kg, Pantothenic acid (min) 125 mg/kg, Biotin (min) 1.25 mg/kg, Choline (min) 1700 mg/kg, Niacin (min) 312 mg/kg, Copper (min) 125 mg/kg, Iron (min) 680 mg/kg, Iodine (min) 8.75 mg/kg, Manganese (min) 937 mg/kg, Selenium (min) 3.75 mg/kg, Zinc (min) 500 mg/kg, Fluorine (max) 370 mg/kg.

The temperatures (maximum and minimum) and relative humidity of the air were recorded twice a day (8 am and 4 pm) by a thermohygrometer. A program of 16 hours of light (natural + artificial) was used throughout the experimental period. The supply of artificial light was controlled by a timer, turning on at 6 pm and turning off at 11 pm, which allowed the lights to be turned on and off during the night, according to the procedure adopted in commercial farms.

For the collection of the frequency of the analyzed behaviors, instant monitoring was performed, with individual visualization of each quail for up to 10

seconds. Thus, during the evaluation of each cage, the number of birds that were expressing certain behavior at that time was noted. The evaluations were carried out twice a week by two evaluators, at the hours of 9:30 am, 10:30 am, 3:30 pm, and 4:30 pm of the day, during the entire experimental period.

Each cage was considered an experimental unit, considering the average of 48 evaluations of each of the four quails observed at four times of the day during the entire experimental period. Observations of each behavior for each experimental unit were calculated as a percentage of birds detected for each behavior pattern (Mohammed et al., 2017).

The main natural behaviors were previously selected from an analysis of birds already submitted to the conditions of the experimental environment. The observed behaviors were adapted from Elston et al. (2000), Barbosa Filho et al. (2007), and Bicas (2017). The behavioral activities reported in the ethogram were quantified (Table 2).

To determine the tonic immobility time, at the end of each period, all birds of the same parcel were

placed in a box, and one quail at a time was abruptly turned over and placed in the supine position on a V-shaped surface according to Jones e Faure (1981). Subsequently, the pressure was placed on the chest for three seconds in each bird before the start of time counting with the aid of a digital stopwatch. To be considered a state of tonic immobility, the bird should remain immobile for at least ten seconds (Heiblum et al., 1998).

**Table 2** - Behavioral ethogram used for laying Japanese quails

<b>Analyzed behavior</b>	<b>Behavior description</b>
Idleness	The moment when the animal does not perform any action, lying or sitting, without demonstration of restlessness.
Comfort	Animal showing signs of comfort, stretching the legs and wings.
Investigate feathers	Animal performing body cleaning and investigating feathers.
Eat	Action in which the animal moves to the food source.
Drink	Action in which the animal moves to the water source.
Cage interaction	Movement through the box or cage, with signs of curiosity.
Agitated	Movement through the box or cage, with signs of unease.
Panting	Action in which the animal demonstrates thermal stress and needs to perform heat exchange by panting.
Aggressive	Act in which an animal invests on another aggressively and with pecking.

### Statistical analysis

The data obtained were analyzed using the statistical software R (R Core Team, 2018), and the normality of the residues was verified through the Shapiro-Wilk test and the homogeneity of variances by Bartlett test. Statistical analysis of non-parametric variables related to the birds' behavior and tonic immobility were submitted to the Kruskal-Wallis test with a 5% significance level.

### Results and discussion

The maximum and minimum temperature found inside the shed during the experimental period was 32.6 °C and 21.7 °C, respectively. The relative humidity varied between 59.6% to 83.5%. The results of room temperature remained above the thermal comfort zone for quails, which should remain around 25.1 °C (Nazareno et al., 2009). On the other hand, the relative

humidity of the air remained adequate, between 57 to 69%, according to what is recommended for quails (Sousa et al., 2014). Quails have a higher tolerance to high temperatures (Umigi et al., 2012), however, when raised under thermally stressful conditions, normal productive performance can be compromised, so it is necessary to guarantee an ideal thermal environment for each age of birds (Sousa et al., 2014).

High temperatures in rearing systems result in a change in the physiological state and changes in the behavioral expression of quails are observed, such as a reduction in trips to the feeder, as well as an increase in idleness and trips to the drinker (Ferreira, 2016), with stress, the main triggering factor for undesirable behaviors such as aggression and feather pecking (Marques et al., 2010).

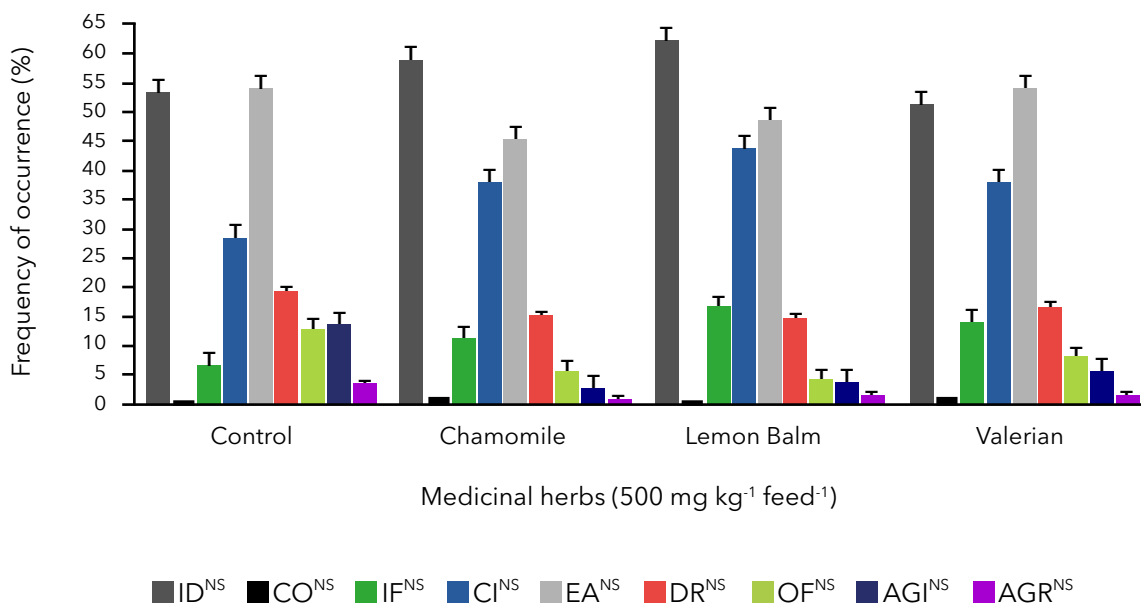
In addition, quails at the peak of production show physiological and hormonal changes that result in biological changes that increase the natural expression of aggressive behaviors. And with advances in nutrition, health, genetics, and management tech-

niques, quail farming has intensified more and more. These changes in current production systems are characterized by an increase in the number of birds per cage, therefore, competition for space and food has become a challenge to quail welfare (Pavan et al., 2005).

In the study, four Japanese quails were housed in each cage measuring 50 cm long, and 38 cm wide, with 475 cm<sup>2</sup> per bird, which is a much higher density than that recommended by Abdel-Azeem (2010),

which is a minimum of 130 cm<sup>2</sup>/bird to guarantee the welfare of the birds, reducing the impact of this environmental factor on the results.

There was no significant difference ( $p > 0.05$ ) between the different medicinal herbs in the behavior of Japanese laying quails according to the Kruskal-Wallis test. However, the control treatment (without herb) in absolute values, showed a higher average of movements of agitated and aggressive behaviors, in comparison to the other treatments (Figure 1).



**Figure 1** - Mean values and standard deviation of the frequency of occurrence of the behaviors, idleness (ID), comfort (CO), investigate feathers (IF), eat (EA), drink (DR), cage interaction (CI), agitated (AGI), anting (PA), aggressive (AGR) of laying Japanese quails fed different herbal medicines in the diet. <sup>NS</sup>Non-significant by the Kruskal-Wallis test ( $p < 0.05$ ).

These results agree with those obtained by Gravena et al. (2009), using valerian extract in the feeding of Japanese quails in the laying phase. The authors concluded that valerian was not able to decrease aggressive behaviors.

Marques et al. (2010) and Silva et al. (2010a) who used other herbal medicines (chamomile and passionflower) with a calming effect on the feeding of laying Japanese quails, did not observe significant differences between the behavioral variables.

Silva (2006) observed that the addition of passion fruit extract to the quail diet in the laying phase was able to decrease the aggressiveness of the birds, reducing the interaction between the birds and the average number of mount behavior, but did not find significant differences for the average number of pecking among birds.

On the other hand, Tenório et al. (2017) state that the inclusion of 1.8 to 5.0 g of chamomile per kg of feed provided for Japanese quails, reduces aggressive

pecking behavior, in addition to keeping the birds sitting for longer. The discrepancies in the results in the literature can be explained by some factors such as the wide variety of species of medicinal plants used, processing of the plants, and the dosages used, which makes it difficult to properly compare studies and the efficiency of medicinal plants. In addition, although herbal medicines are agents with great action potential, the number of studies is still low.

Tonic immobility is a defensive behavior used as the last anti-predatory defense response of some species (Michelan et al., 2006) and is preceded primarily by coping behavior and nervous system responses to a stress situation (Marques et al., 2010). The animal exhibits an immobile posture pretending to be dead to get an opportunity to escape, as it induces the relaxation of the predator's attention (Michelan et al., 2006).

According to the Kruskal-Wallis test, there is at least one significant difference ( $p < 0.05$ ) in the distribution of tonic immobility in birds evaluated with different herbal medicines in the diet (Table 3). The chamomile-containing diet provided the shortest time in seconds in tonic immobility and the control treatment the longest time in seconds.

**Table 3** - Time (seconds) in tonic immobility of laying Japanese quails fed different herbal medicines in the diet

Medicinal herbs	Time in tonic immobility
Control	21.60
Chamomile <sup>1</sup>	8.27
Lemon Balm <sup>1</sup>	11.51
Valerian <sup>1</sup>	13.87
CV (%)	40.82
p-value <sup>2</sup>	0.004

Note: <sup>1</sup>500 mg/kg feed; CV = coefficient of variation; <sup>2</sup>Values > 0.05 are considered non-significant.

The main bioactive properties of chamomile are found in the floral chapters, which is the part of the plant that is most used for medicinal purposes. More than 120 chemical constituents have been identified as secondary metabolites, being: 28 terpenoids, 36

flavonoids, and 52 other compounds with pharmacological potential (Buono-Core et al., 2011). Among these chemicals, apigenin is an important flavonoid isolated from the dehydrated chamomile flower.

Natural flavonoid compounds have an affinity as ligands for  $\gamma$ -Aminobutyric acid type A receptors (GABA<sub>A</sub>) in the central nervous system region, being able to model chloride currents generated by GABA, positively or negatively (Fernández et al., 2006). In addition, flavonoids have already demonstrated the ability to modulate the serotonergic pathways and the hypothalamic-pituitary-adrenal axis, exerting an anxiolytic and sedative function (Jian et al., 2016) and can act in more than one pathway.

The presence of this substance in the diet made chamomile the herb with the greatest effect on the tonic immobility time of laying Japanese quails. However, the data obtained by this study demonstrate that the birds that had dietary lemon balm (*Melissa officinalis* L.), valerian (*Valeriana officinalis* L.) 500 mg/kg feed also obtained lower mean tonic immobility times in relation to treatment without herbal medicine, also due to its anxiolytic and calming properties (Yuan et al., 2004).

The results found in this study are consistent with those found by Silva et al. (2010a) using dry extract of passiflora leaves (*Passiflora alata*), Silva et al. (2010b) using Kava (*Piper methysticum*) for laying quails, both reported a decrease in tonic immobility time in quails. The time in tonic immobility is considered a natural behavior related to stress, that is, the more stressed the bird is, the longer the time in this state will be (Heiblum et al., 1998; Savory et al., 1999).

The results found in this study using the dosage of 500 mg/kg feed of herbal medicines, did not confirm the calming and anxiolytic characteristics present in the extract of the herbs chamomile, lemon balm, and valerian on the behavior of birds. It is possible to affirm that the dosages used were not able to modulate the stress and beneficially influence the behavior of the laying Japanese quails and therefore also did not positively impact the performance of the birds.

The number of studies on herbal medicine is still low, with a large discrepancy in the results. Therefore, further studies are needed using herbal medicines with doses higher than those used in this study, to improve the welfare of animals, especially in Japanese quails.

## Conclusion

The use of chamomile, lemon balm, and valerian herbs (500 mg/kg feed) did not influence the behavior of laying Japanese quails, however, they were able to decrease the tonic immobility time of the birds, with chamomile being the herb that exercised greater effect.

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