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Inclusion of lemon balm (*Lippia alba*) as a phytogenic additive in the diet of Japanese quail

Inclusão de erva-cidreira (*Lippia alba*) como aditivo fitogênico na alimentação de codornas japonesas

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Highlights

The inclusion of lemon balm influenced the performance of Japanese quail.
Including lemon balm in the feed affected the quality of quail eggs.
The addition of lemon balm did not influence the leukocyte count in quail.

Abstract

Japanese quail is a small bird with a short production period. However, the use of performance-enhancing antibiotics can leave residues in its meat and eggs. As an alternative, phytogenic additives can be used to cultivate beneficial bacteria in the gastrointestinal tract, improving the digestion and absorption of ingested nutrients. This study aimed to evaluate the effects of adding different concentrations of lemon balm as a phytogenic additive on the performance, egg quality, and differential leukocyte count of Japanese quail raised in the semiarid region of Brazil. The experiment was conducted in the poultry sector of the Paraíba Institute of Science and Technology – Sousa-PB campus, from June to September 2022. Eighty Japanese quail, 80 days old and standardized by production and body weight, were utilized. The design was completely randomized with four treatments and four replicates, each containing five birds per plot. The experimental period was 88 days, consisting of four 22-day cycles. Dried lemon balm

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was incorporated as a phytogenic additive in the birds' diet at concentrations of 0, 0.3, 0.6, and 0.9%. Performance variables and egg quality were assessed. Regression analysis was conducted for statistical analysis using SAS software. To determine the differential leukocyte count, two blood samples were collected and analyzed with blood smears. The leukocyte counts underwent analysis of variance and Tukey's test, at a significance level of 5%, using the statistical program R. A significant decreasing linear effect was observed for feed conversion per dozen eggs, as well as a quadratic effect for conversion per egg mass. Albumen height showed a significant quadratic effect, and there was a decreasing linear effect on albumen percentage. Additionally, a quadratic effect was noted for Haugh Unit. The inclusion of lemon balm had a positive effect on feed conversion per dozen eggs and enhanced albumen height, suggesting a requirement of 0.501% for optimal results, as well as on yolk color, which showed an intensified shade with increased herb inclusion. The Haugh Unit also demonstrated a significant effect, with an optimal concentration of 0.495%. The use of 0.5% dried lemon balm (*Lippia alba*) is recommended as a phytogenic additive for Japanese quail in the laying phase. The addition of different concentrations of dried lemon balm did not influence the differential leukocyte count or the heterophil:lymphocyte ratio of Japanese quail in the semiarid region.
Key words: Egg production. Hematology. Herbal extract. Yolk index. Quail farming.

Resumo

A codorna japonesa uma ave de pequeno porte e com curto período de produção. Contudo, o uso de antibióticos melhoradores de desempenho pode deixar resíduos na carne e nos ovos. Como alternativa, aditivos fitogênicos podem ser utilizados para o desenvolvimento de bactérias benéficas no trato gastrointestinal para melhorar a digestão e absorção de nutrientes ingeridos. Objetivou-se avaliar o efeito da adição de diferentes concentrações de erva-cidreira, como aditivo fitogênico, sobre desempenho, qualidade de ovos e contagem diferencial de leucócitos, de codornas japonesas criadas no semiárido. O experimento foi realizado no setor avícola do Instituto de Ciência e Tecnologia da Paraíba – campus Sousa-PB, no período de junho a setembro de 2022. Foram utilizadas 80 codornas japonesas com 80 dias de idade, padronizadas por produção e peso corporal. O delineamento utilizado foi o inteiramente casualizado com quatro tratamentos e quatro repetições contendo cinco aves por parcela. O período experimental durou 88 dias em quatro ciclos de 22 dias. A erva-cidreira desidratada foi utilizada como aditivo fitogênico em quatro tratamentos na alimentação das aves (0%; 0,3%; 0,6% e 0,9%). Foram avaliados variáveis de desempenho e qualidade dos ovos. A análise de regressão foi utilizada para análise estatística das mesmas, utilizando-se o programa SAS. Para determinação da contagem diferencial leucocitária, foram realizadas duas coletas de sangue com a realização do esfregaço sanguíneo. As contagens diferenciais de leucócitos foram submetidas a análise de variância e teste de Tukey, ao nível de significância de 5%, com o auxílio do programa estatístico R. Houve efeito linear decrescente significativo para a conversão alimentar por dúzia de ovos, bem como efeito quadrático para a conversão por massa de ovos. Houve efeito quadrático significativo para a altura do albúmen e linear decrescente para porcentagem de albúmen. Houve efeito quadrático para a Unidade Haugh. Observou-se efeito positivo da inclusão da erva-cidreira sobre a conversão alimentar por dúzia de ovos e efeito benéfico sobre a altura do albúmen, sendo possível estimar a exigência em 0,501%, bem como sobre a coloração da gema, observando-se maior tonalidade a partir da maior inclusão da erva. Houve efeito significativo para a Unidade Haugh sendo possível estimar a exigência em 0,495%. Recomenda-se a utilização de 0,5% de erva-cidreira (*Lippia alba*) desidratada como aditivo fitogênico para codornas japonesas na fase de postura. A adição de diferentes concentrações de erva-cidreira desidratada, não influenciou a contagem diferencial leucocitária e a relação heterofilos: linfócitos de codornas japonesas no semiárido.

Palavras-chave: Coturnicultura. Extrato herbal. Hematologia. Índice de gema. Produção de ovos.

Introduction

The Japanese quail (*Coturnix coturnix japonica*) is a small bird with a short production period and is considered an attractive alternative to meet the growing demand for animal protein (Cullere et al., 2018). Attributes making this venture appealing include the birds' small size, adaptability, suitability for raising in limited spaces, and relatively low startup costs. The habits and management of quail are similar to those of chickens, yet they require less space for reproduction and egg laying, consume less food compared to chickens, and can be housed in small poultry sheds in groups.

In the poultry industry, the inclusion of performance-enhancing antibiotics in diets at subtherapeutic doses is a common practice that dates back to the 1940s (Gadde et al., 2018). However, there is a global shift towards eliminating these substances from animal feed due to concerns over residue in meat and eggs and the risk of bacterial resistance.

Phytogenic additives, which include herbal and spice derivatives, serve a role similar to antibiotics by promoting the growth of beneficial bacteria in the gastrointestinal tract, thereby enhancing nutrient digestion, absorption, and overall bird health and performance (Fernandes et al., 2015).

Plant-derived additives are garnering significant interest in animal feed research. Characterized by their low toxicity and residue-free qualities, these substances are increasingly preferred over synthetic antibiotics (Madhupriya et al., 2018). Lemon balm, a plant commonly used as a phytotherapeutic, shows promising potential for research as a phytogenic additive.

Lippia alba, also known as lemon balm, belongs to the family Verbenaceae and is prevalent in South America, Central America, and Africa. Noted for its rich phytochemical variety, particularly in the terpene group, it is extensively used in traditional medicine to address gastrointestinal and respiratory ailments. Additionally, it is applied as an antispasmodic, antipyretic, anti-inflammatory, antidiarrheal, analgesic, and sedative agent (Peixoto et al., 2015).

Given the significant potential of *Lippia alba*, further biological studies are essential to explore the various indications of this plant. Thus, this study aims to assess the impact of varying concentrations of *Lippia alba* as a phytogenic additive on the productive performance, egg quality, and differential leukocyte count of laying Japanese quail in the semiarid region.

Material and Methods

The experiment was conducted at the Federal Institute of Science and Technology of Paraíba, on the Sousa-PB campus. The municipality is situated at an altitude of 220 m, with coordinates at latitude 06º45'33" S and longitude 38º13'41" W. The local climate is classified as hot and dry (BSh type according to the Köppen classification), with an average annual adjusted temperature of 26.6 ºC and an annual precipitation of 1050.2 mm (Instituto Nacional de Meteorologia [INMET], 2010). The study was carried out between June and September 2022.

The research project received ethical clearance from the Animal Research Ethics Committee of the institution, under approval number 01.0462.2017.

A completely randomized design was utilized, consisting of four treatments and four replicates, each containing five birds per plot. The experimental duration was 88 days, divided into four cycles of 22 days each.

Eighty Japanese quail (*Coturnix coturnix japonica*), 80 days old and standardized by production and body weight (average 315.6 g), were used. The birds were housed in galvanized wire cages measuring 50 cm × 35 cm, equipped with pressure-type chick drinkers and trough feeders.

Throughout the experimental period, all birds were subjected to similar handling and feeding conditions, with water and feed available *ad libitum.*

Dried lemon balm (*Lippia alba*) was incorporated as a phytogenic additive into the birds' feed at four different levels (0, 0.3, 0.6, and 0.9%).

The lemon balm was harvested in the rural areas of Sousa and Patos - PB. The plant shoots were pre-dried in a forced air circulation oven at 55 °C for 72 h to ascertain the partial dry matter content. Following pre-drying, the samples were milled using a Wiley-type mill with 1-mm sieves. The resulting lemon balm powder was then stored in hermetically sealed glass bottles, labeled for subsequent analysis of final dry matter (DM), crude protein (CP), organic matter (OM), mineral matter (MM), and ether extract (EE), following the methodology described by D. J. Silva and Queiroz (2002). Analysis of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was conducted according to the methodology proposed by Van Soest (1994). These analyses were performed in the animal nutrition laboratory of the IFPB – Sousa campus (Table 1).

The diets were formulated to meet the nutritional requirements of laying Japanese quail, following the recommendations by J. H. V. Silva and Costa (2009).

Table 2 details the composition and calculated nutritional values of the diets used during the four egg production cycles.

Table 1
Chemical composition of dried lemon balm (*Lippia alba*), used in feed for Japanese quail

DM (%)	OM (%)	MM (%)	TN (%)	CP (%)	NDF (%)	ADF (%)	EE (%)
91.39	93.47	6.53	0.42	2.64	61.76	50.36	1.01

Dry matter (DM), organic matter (OM), total nitrogen (TN), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), mineral matter (MM) and ether extract (EE).

Table 2
Chemical composition and calculated nutritional levels in diets based on corn and soybean meal with the inclusion of 0, 0.3, 0.6, and 0.9% dried lemon balm

Ingredient (g kg^{-1})	Lemon balm inclusion level			
	0%	0.3%	0.6%	0.9%
Corn	53.721	53.677	53.057	52.437
Soybean meal	33.420	33.028	33.132	33.236
Limestone	5.506	5.503	5.501	5.499
Mineral mixture[1]	4.000	4.000	4.000	4.000
Soybean oil	2.195	2.299	2.513	2.726
Meat and bone meal	0.339	0.356	0.362	0.367
Common salt	0.326	0.326	0.327	0.327
Sodium bicarbonate	0.276	0.276	0.276	0.276
DL-methionine	0.146	0.150	0.151	0.152
L-lysine	0.071	0.083	0.081	0.079
Lemon balm	0	0.300	0.600	0.900
Total (kg)	**100.00**	**100.00**	**100.00**	**100.00**
NUTRIENT				
Calcium (g kg^{-1})	30.500	30.500	30.500	30.500
Chlorine (%)	0.2400	0.2400	0.2400	0.2400
Metabolizable energy (kcal kg^{-1})	2800.0000	2800.0000	2800.0001	2800.0000
Available phosphorus (%)	0.2800	0.2800	0.2800	0.2800
Digestible lysine (%)	1.0300	1.0300	1.0300	1.0300
Digestible methionine + cystine (%)	0.7000	0.7000	0.7000	0.7000
Crude protein (%)	20.000	19.8422	19.8376	19.8330
Sodium (%)	0.2300	0.2300	0.2300	0.2300
Digestible threonine	0.6761	0.6700	0.6700	0.6700

[1] Moisture (max) 130.00 g kg^{-1}, Crude protein (min) 250.00 g kg^{-1}, Ether extract (min) 40.00 g kg^{-1}, Crude fiber (min) 2,000.00 mg kg^{-1}, Mineral matter (max) 700.00 g kg^{-1}, Calcium (min) 130.00 g kg^{-1}, Calcium (max) 150.00 g kg^{-1}, Phosphorus (min) 45.00 g kg^{-1}, Sodium (min) 33.00 g kg^{-1}, Iron (min) 640.00 mg kg^{-1}, Cobalt (min) 4.30 mg kg^{-1}, Vitamin A (min) 160,000.00 IU kg^{-1}, Vitamin D3 (min) 40,000.00 IU kg^{-1}, Vitamin E (min) 300.00 IU kg^{-1}, Vitamin K3 (min) 39.80 mg kg^{-1}, Vitamin B1 (min) 19.80 mg kg^{-1}, Vitamin B2 (min) 80.00 mg kg^{-1}, Niacin (min) 199.90 mg kg^{-1}, Pantothenic acid (min) 199.90 mg kg^{-1}, Vitamin B6 (min) 59.78 mg kg^{-1}, Vitamin B12 (min) 160.00 mcg kg^{-1}, Choline (min) 5,370.00 mg kg^{-1}, Methionine (min) 15.50 g kg^{-1}, Lysine (min) 12.20 g kg^{-1}, Phytase (min) 5,000.00 ftu kg^{-1}.

The lighting regimen comprised 17 h of light per day (12 natural and 5 artificial) throughout the experimental duration.

For bioclimatic characterization, readings were taken every 10 min over the 88-day evaluation period, using data loggers, to measure the Temperature-Humidity Index (THI) and the Black Globe Humidity Index (BGHI).

The equipment was installed at the height of the cage row, oriented towards the birds. Measurements included room temperature, black globe temperature, and relative humidity. Black globe temperature was measured using black globe thermometers connected to a data logger, while relative humidity was monitored using a separate data logger.

The two bioclimatic indices, THI and BGHI, were calculated using the equations below:

$$THI = 0.72\ (t_{db} + t_{wb}) + 40.6,$$

where t_{db} = dry bulb temperature (°C); and t_{wb} = wet bulb temperature (°C), as per Thom (1959).

$$BGHI = t_{bg} + 0.36\ (t_{dp}) + 41.5,$$

where t_{bg} = black globe temperature (°C); and t_{dp} = dew point temperature (°C), according to Buffington et al. (1981).

The averages of the bioclimatic variables were determined for the following times: dawn (00:00 to 06:00), morning (06:01 to 12:00), afternoon (12:01 to 18:00), and night (18:01 to 23:59) (Table 3).

Table 3
Dry bulb temperature (DBT), relative humidity (RH), temperature-humidity index (THI), and black globe humidity index (BGHI) values obtained during an experiment with Japanese quail fed diets containing different concentrations of lemon balm

TIME	DBT	RH	THI	BGHI
DAWN – 00:00 to 06:00	23.728	69.623	71.865	71.851
MORNING – 06:01 to 12:00	26.190	62.674	74.722	75.338
AFTERNOON – 12:01 to 18:00	30.931	47.835	78.974	80.266
NIGHT – 18:01 to 23:59	26.887	59.059	75.243	75.838

To analyze the performance of the birds, the following metrics were evaluated: Feed intake was calculated by the difference between the quantity of feed provided and the leftovers. Feed conversion by the mass of eggs produced was determined by dividing the total feed consumed by the weight of the eggs produced, expressed as grams of feed per gram of egg (g/g). Feed conversion per dozen eggs was calculated by averaging the feed intake (kg) and dividing by 12.

The laying percentage and commercial viability were calculated by counting the number of whole, broken, cracked, thinshelled, shell-less, and deformed eggs twice daily, and recording them on specific worksheets.

To assess egg quality, three whole eggs were collected from each plot on the last three days of each phase of the experiment to evaluate external and internal quality parameters.

External quality was determined by the following parameters: Average egg weight was calculated using the total weight measured on a 0.01-g precision scale, divided by the number of eggs per plot. Shell weight was measured after breaking the shells in half, including the internal membrane, washing under running water, drying at room temperature (24 to 48 h), and weighing again on a 0.01-g precision scale. Shell thickness, including the membranes, was measured at three different points (both poles and the side) using a digital caliper with a precision of 0.01 mm. External quality was assessed based on the specific gravity of all whole eggs produced per plot, which were immersed in a saline solution. This variable was measured using a petroleum densimeter with a density range of 1.060 to 1.085 g/mL (Garcia et al., 2010).

Internal quality was assessed by the following: Yolk weight was determined by manually separating the yolks from randomly broken eggs and weighing them on a 0.01-g precision scale. Albumen weight was calculated as the difference between the total egg weight and the combined weights of the shell and yolk. Yolk height and diameter were measured using a digital caliper. Yolk index was calculated as the ratio of yolk width to height (F. H. A. Silva, 2004). Yolk color was determined using a Roche® colorimetric fan (score from 1 to 16). Yolk percentage was calculated based on the total egg weight and yolk weight. Albumen height and diameter were also measured using a digital caliper with 0.01-mm precision. Albumen percentage was calculated as 100 − (% yolk + % shell) (Santos et al., 2009). Haugh Unit was calculated using the formula: $HU = 1\ mm \times \log (H - 1.7\ W^{0.37} + 7.6)$, where H is the height of the albumen (mm) and W is the weight of the whole egg (g).

To determine the differential leukocyte count, two blood samples were collected, the first on 07/28/2022 and the second on 09/05/2022. Blood was drawn from the medial metatarsal vein using 1-mL syringes, from one bird in each plot. Differential leukocyte counts were performed at the Veterinary Clinical Pathology Laboratory of the Veterinary Hospital of the IFPB Sousa - PB Campus.

In the differential leukocyte count, blood smears were prepared and stained with Panoptic dye (Laborclin-Laboratory Supplies Ltd., Pinhais-PR, Brazil). The cells were identified under a microscope using an oil immersion objective (100X) following the method described by Thrall (2015). Leukocytes were classified based on their morphological and staining characteristics, and results were expressed as a percentage of each cell type.

The heterophil:lymphocyte ratio was calculated by dividing the number of heterophils by the number of lymphocytes.

The differential leukocyte count and heterophil:lymphocyte ratio data were subjected to analysis of variance and Tukey's test, at a significance level of 5%, using R statistical software (R Core Team [R], 2023).

For the statistical evaluation of egg performance and quality, regression analysis was employed via SAS statistical software (Statistical Analysis System Institute [SAS Institute], 2011). This analysis aimed to determine the optimal level of additive inclusion based on the R^2 value, which reflects the biological response of the birds.

Results and Discussion

Table 4 details the effects of increasing dietary levels of lemon balm on the performance and egg production of Japanese quail.

Table 4
Effect of treatments on laying rate (%), dirty eggs (%), cracked eggs (%), feed intake (g/bird/day), feed conversion per dozen eggs (kg/dozen), and feed conversion per egg mass (kg kg^{-1}) in Japanese quail fed diets with different concentrations of *Lippia alba*

Variable	Lemon balm inclusion (%)				Equation
	0	0.3	0.6	0.9	
Laying rate (%)	84.0253	87.0557	88.8123	90.1791	
Dirty eggs (%)	1.4886	1.1779	0.8408	1.2639	
Cracked eggs (%)	0.8489	0.8995	1.0577	0.1692	
Feed intake (g/bird/day)	30.9010	31.8104	30.4648	31.3063	
Feed conversion per dozen eggs (kg/dozen)	0.4361	0.4175	0.3956	0.3940	$y = -0.0495x + 0.433$ $R^2 = 0.9166$
Feed conversion per egg mass (kg kg^{-1})	2.3206	2.2934	2.7434	2.2975	$y^2 = -1.163x^2 + 1.1736x + 2.252.$ $R^2 = 0.3514$

The performance data (Table 4) showed a significant decreasing linear effect (P<0.01) on feed conversion per dozen eggs ($y = -0.0495x + 0.433$, $R^2 = 0.9166$) (Figure 1), and a quadratic effect (P<0.05) on feed conversion per egg mass ($y^2 = -1.163x^2 + 1.1736x + 2.252$, $R^2 = 0.3514$). These results suggest an optimal dietary inclusion level of lemon balm as a phytogenic additive at 0.504%.

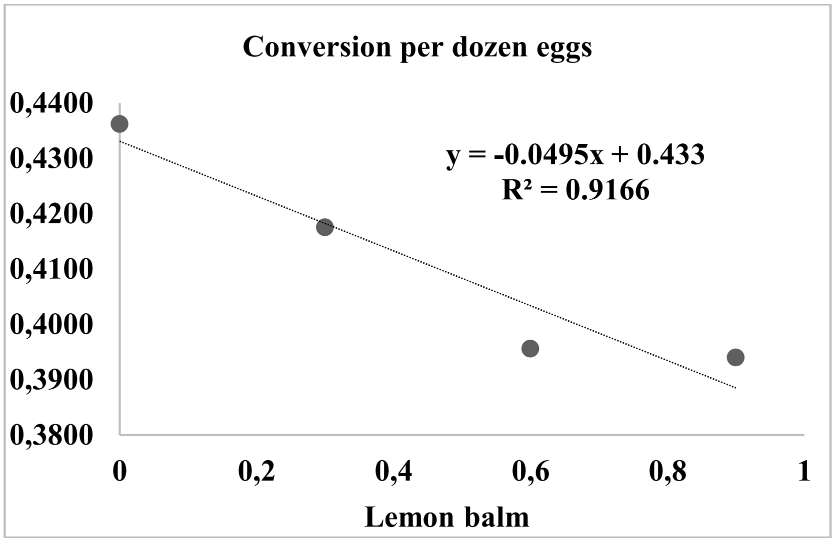

Figure 1. Effect of including lemon balm in quail feed on feed conversion per dozen eggs.

The potential mechanism behind the efficacy of these botanical components may involve their antioxidant properties, which enhance feed utilization, thereby improving the digestibility of energy and protein and optimizing quail egg production performance (Sultan et al., 2015).

Laying rate (%), dirty eggs (%), cracked eggs (%), and intake (g/bird/day) showed no statistically significant effects (Table 4).

Table 5 describes the effect of increasing dietary levels of lemon balm on egg quality.

There was a significant quadratic effect ($P<0.05$) on albumen height ($y = -1.3848x^2 + 1.3897x + 5.1263$; $R^2 = 0.869$), indicating an optimal requirement of 0.501%. This suggests that lemon balm supplementation can enhance egg quality.

The beneficial effects on feed conversion could improve the nutritional supply for the turnover of albumen-secreting epithelial cells in the magnum. Additionally, lemon balm may protect the magnum epithelium through its antibacterial properties, which could inhibit oviduct colonization by pathogenic bacteria.

Significant increasing linear effects were noted for yolk color ($P<0.01$) (Figure 2) and yolk diameter ($P<0.05$).

The carotenoids present in the diet, influenced by the inclusion of lemon balm, affect yolk color intensity (Seibel et al., 2010).

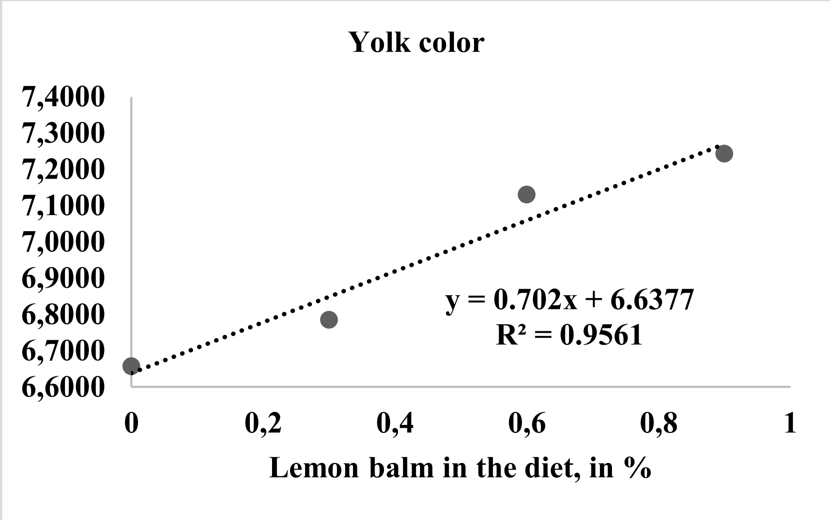

Figure 2. Effect of including lemon balm in quail feed on yolk color.

Table 5
Effect of treatments on egg quality variables of Japanese quail fed diets with different concentrations of *Lippia alba*

Variable	Lemon balm inclusion (%)				Equation
	0	0.3	0.6	0.9	
Egg weight (g)	13.3045	13.1582	13.4336	13.5063	
Albumen height (mm)	5.1031	5.4881	5.3921	5.2785	y = -1.3848 x2 + 1.3897x + 5.1263; R2 = 0.869
Albumen weight (g)	7.2268	7.2120	7.1009	7.1299	
Yolk diameter (mm)	5.2167	5.3485	5.4018	5.4387	y = 0.6844x + 24.539; R2 = 0.684
Yolk height (mm)	24.5058	24.6638	25.2106	25.0080	
Yolk color	6.6563	6.7847	7.1302	7.2431	y = 0.702x +6.6377; R2 = 0.956
Shell weight (g)	1.1412	1.1305	1.1382	1.1290	
Shell thickness (mm)	0.4591	0.4674	0.4780	0.4686	
Albumen (%)	54.2903	53.4631	52.8275	52.7514	y = -1.7508 x + 54.121; R2 = 0.903
Yolk (%)	39.3842	39.7256	39.8660	39.9924	
Shell (%)	8.7098	8.5566	8.4930	8.3633	
Specific gravity	1.0715	1.0700	1.0684	1.0689	
Yolk index	0.4117	0.4080	0.3881	0.3982	y = -0.0201x + 0.4105 R^2 = 0.5423
Haugh Unit	91.7642	93.6282	93.1797	92.5454	y = -6.9398x2 + 6.8775x + 91.871 R^2 = 0.885

A significant effect was noted on the yolk index (Table 5), which decreased linearly ($P<0.05$) ($y = -0.0201x + 0.4105$; $R^2 = 0.542$). According to Genchev (2012), the yolk index of fresh eggs typically ranges from 0.38 to 0.52, which is similar to findings from this study, indicating that the quality of the yolk was maintained despite the reduced yolk index.

A decreasing linear effect was also observed for the albumen percentage ($P<0.05$) ($y = -1.7508x + 54.121$; $R^2 = 0.903$) (Table 5), possibly due to an increase in yolk percentage.

A significant quadratic effect ($P<0.05$) was found for the Haugh Unit ($y = -6.9398x^2 + 6.8775x + 91.871$; $R^2 = 0.885$), allowing the requirement to be estimated at 0.495%. This outcome aligns with the expected results, as the Haugh Unit value is derived from a mathematical equation relating egg weight to albumen height.

Haugh Unit, an important measure of egg internal quality, indicates that eggs with a value above 72 are of excellent quality, according to the United States Department of Agriculture [USDA] (2000). In this study, all eggs exceeded this threshold.

No statistically significant effects were observed for egg weight (g), albumen weight (g), yolk height (mm), shell weight (g), shell thickness (mm), yolk (%), shell (%), or specific gravity.

Table 6 presents the average relative values (%) of lymphocytes, eosinophils, heterophils, monocytes, basophils, and the heterophil:lymphocyte (H:L) ratio in Japanese quail fed diets with varying concentrations of lemon balm.

As indicated in Table 6, no significant differences ($p>0.05$) were observed between the treatment groups and control across the variables of heterophils, lymphocytes, monocytes, eosinophils, and the H:L ratio.

The study found that relative lymphocyte values were lower than the range of 50% to 70% typically observed in quail, as suggested by Thrall (2015). These results do not appear to be influenced by the inclusion of lemon balm in the diet, as the lymphocyte levels across different treatment levels were not significantly different ($p<0.05$) from those in the control group.

Nordi (2007) suggests that the number of lymphocytes decreases in birds experiencing stress due to corticosterone release. The observed variations in lymphocyte counts may have been influenced by heat stress experienced by the animals in this study.

Similarly, Porto and Fontenele (2020) found that chronic heat stress led to a reduction in lymphocyte numbers in Japanese quail exposed to high ambient temperatures, indicating that prolonged heat stress after ten days of age can cause immunosuppression.

There was a significant difference ($p<0.05$) in relative basophil counts between the treatments, with values exceeding the 0% to 2% range considered normal for the species by Thrall (2015). However, this increase is likely not attributable to the lemon balm in the diet, as the basophil levels in quail fed the highest inclusion rate of lemon balm did not significantly differ ($p<0.05$) from those in the control group.

The relative basophil counts found in this study can be attributed to stress during capture and blood collection and in response to high temperatures during the experiment.

Table 6
Relative values (%) of leukocyte variables and heterophil:lymphocyte ratio of Japanese quail fed diets containing different concentrations of lemon balm

Leukocyte variable	Collection	Lemon balm inclusion (%)			
		0	0.3	0.6	0.9
Heterophils %	1	39 ± 1.08	47.75 ± 5.09	39.75 ± 3.17	40 ± 1.22
	2	42 ± 0.91	43.25 ± 1.49	40.25 ± 1.44	41.25 ± 2.5
	Mean	40.5 ± 0.87	45.5 ± 2.6	40 ± 1.61	40.62 ± 1.31
Lymphocytes%	1	50.25 ± 1.89	44 ± 4.38	47.5 ± 2.18	48.5 ± 1.66
	2	47.75 ± 0.75	49 ± 0.41	48.75 ± 1.49	48.75 ± 2.5
	Mean	49 ± 1.05	46.5 ± 2.24	48.12 ± 1.25	48.62 ± 1.39
Monocytes%	1	2.25 ± 0.48	1.25 ± 0.48	2 ± 0.41	2.75 ± 0.63
	2	3.75 ± 0.63	2.75 ± 0.63	3.75 ± 0.48	2.75 ± 1.18
	Mean	3 ± 0.46	2 ± 0.46	2.88 ± 0.44	2.75 ± 0.62
Basophils %	1	3 ± 0.71	2.25 ± 0.63	3 ± 0.91	4.75 ± 0.25
	2	3.5 ± 0.87	2 ± 0.41	3.75 ± 0.85	3.75 ± 1.03
	Mean	3.25 ± 0.53[AB]	2.12 ± 0.35[B]	3.38 ± 0.6[AB]	4.25 ± 0.53[A]
Eosinophils %[a]	1	15.75 ± 9.72	4.75 ± 0.75	7.75 ± 5.09	4 ± 0.41
	2	2.5 ± 0.5	3.25 ± 0.25	3.5 ± 1.19	3.5 ± 0.96
	Mean	9.12 ± 5.15	4 ± 0.46	5.62 ± 2.55	3.75 ± 0.49
Heterophil:Lymphocyte ratio[b]	1	0.78 ± 0.04	1.17 ± 0.29	0.83 ± 0.04	0.83 ± 0.05
	2	0.88 ± 0.03	0.88 ± 0.04	0.83 ± 0.05	0.86 ± 0.08
	Mean	0.83 ± 0.03	1.03 ± 0.14	0.83 ± 0.03	0.84 ± 0.05

[a] Original means; however, the analysis of variance was executed with transformed data using Box-Cox transformation with a Lambda parameter equal to -0.55 to correct non-normality of residuals.
[b] Original means; however, the analysis of variance was executed with transformed data using the Box-Cox transformation with a Lambda parameter equal to -2 to correct heteroscedasticity.
[A, B] Different uppercase letters in the same row represent a significant difference ($p<0.05$), for the treatments.

According to Thrall (2015), the role of basophils in birds remains unclear. It is believed that they are involved in acute inflammatory reactions and type IV hypersensitivity. Additionally, Kokosharov (1998) and Davis et al. (2008) suggest that basophil levels in birds may rise under conditions of severe stress, whether acute or prolonged, and in response to toxic and septicemic processes.

Rosa et al. (2011) assessed stress levels in quail during production cycles at various temperatures, monitoring changes in the leukogram and the H:L ratio. They found no correlation between basophilia and heat stress during the experiment, although the highest temperature recorded was 25.5 °C.

Although the H:L ratio is widely considered the most sensitive stress indicator in birds (Davis et al., 2008), Maxwell

(1993) noted that both heteropenia and basophilia could occur during severe stress. In his study, basophilia was present in all treatment groups.

The relative values of the other leukocyte cells are within the reference values described by Thrall (2015).

The relative values of other leukocyte cells remained within the reference ranges defined by Thrall (2015).

In the current study, the mean H:L ratios for all treatments exceeded the reference values indicative of high stress. This finding seems unrelated to the inclusion of lemon balm in the diet, as there were no significant differences (p<0.05) compared to the control group.

Conversely, Nazar et al. (2019) found that dietary thymol supplementation in Japanese quail exposed to heat stress positively affected the H:L ratio, an improvement attributed to the antioxidant and immunostimulant properties of thymol.

In this study, an increased H:L ratio indicated an environment detrimental to the birds' well-being. Despite these findings, the birds exhibited no clinical signs of disease and maintained production rates exceeding 84% across all treatments. The increase likely responded to heat stress.

Corroborating these findings, Porto and Fontenele (2020) observed higher means for H:L ratio in quail raised under heat stress conditions. From day 20 onward, the authors noted reduced absolute weights of the liver, heart, cloacal bursa, and bursal follicles, along with heterophilia, lymphopenia, and an elevated H:L ratio.

Conclusions

The inclusion of lemon balm (*Lippia alba*) up to 0.9% as a phytogenic additive in the diet of Japanese quail during the laying phase improved feed conversion per dozen eggs and enhanced yolk color.

Adding up to 0.9% dried lemon balm as a phytogenic additive to the diet did not affect the differential lymphocyte count or the heterophil:lymphocyte ratio in Japanese quail during the laying phase, raised in the semiarid region.

Observations of basophilia, lymphopenia, and heterophil:lymphocyte ratios in the treatments indicate an increase in stress levels in Japanese quail maintained at room temperature in the semiarid region.

The use of 0.5% dried lemon balm as a phytogenic additive is recommended for Japanese quail in the laying phase.

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