



Moringa Oleifera: an alternative ingredient to improve the egg quality of Japanese quail

[*Moringa oleifera*: um ingrediente alternativo na melhora da qualidade de ovos de codornas japonesas]

R.G. Garcia¹, E.R.S. Gandra³, M.F.C. Burbarelli^{1*}, J.K. Valentim¹, G.A. Felix²,
B.A. Lopes², F.B. Bacha², C.M.F. Melo⁴, J.P. Silva²,
C.M. Komiyama¹, F.R. Caldara¹

¹Aluno de pós-graduação - Universidade Federal de Grande Dourados - Dourados, MS

²Centro Universitário da Grande Dourados - Dourados, MS

³Universidade Federal do Sul e Sudeste do Pará - Xinguara, PA

⁴Universidade Estadual Paulista - Jaboticabal, SP

R.G. Garcia
<https://orcid.org/0000-0002-4978-9386>
E.R.S. Gandra
<https://orcid.org/0000-0002-4565-2817>
M.F.C. Burbarelli
<https://orcid.org/0000-0002-8079-2890>
J.K. Valentim
<https://orcid.org/0000-0001-8547-4149>
G.A. Felix
<https://orcid.org/0000-0002-7739-586X>
B.A. Lopes
<https://orcid.org/0000-0003-1560-7676>
F.B. Bacha
<https://orcid.org/0000-0002-1476-8921>
C.M.F. Melo
<https://orcid.org/0000-0001-6347-6012>
J.P. Silva
<https://orcid.org/0000-0001-7715-6255>
C.M. Komiyama
<https://orcid.org/0000-0001-8732-5745>
F.R. Caldara
<https://orcid.org/0000-0002-7564-6127>

ABSTRACT

This study aims to evaluate effects of inclusion of moringa in Japanese quail diets on laying performance, egg quality, blood parameters, serum biochemical profile, and behavior. One hundred and forty-four Japanese quails, approximately 35-d-old, were distributed in a completely randomized design with four treatments and six replications. Treatments were diet inclusion of 0, 2, 4 and 6% of dried and ground moringa leaves. The inclusion of moringa in diets reduces the quadratic feed intake up to the level of 1.20%, increases weight of eggs with a quadratic behavior up to 3.80%, and linearly increases yolk weight. Yolk color changes with higher levels of inclusion of moringa and resulted in more intense colors. The biochemical profile of quails changed slightly but remains within the normal range. The inclusion of 4% of moringa in diets increases alkaline phosphatase. Regarding cholesterol and triglycerides, diet with 6% moringa inclusion was lower when compared to the others. The behavior of laying quails does not change due to inclusion of moringa in diets. Up to 3.83% of *Moringa oleifera* can be included in Japanese quail diet to improve egg quality without compromising performance, biochemical profile, blood parameters and behavior.

Keywords: antioxidants, egg quality, laying quails, medicinal plants, phytogetic additives

RESUMO

O objetivo deste estudo foi avaliar a inclusão de *Moringa oleifera* na dieta de codornas japonesas sobre o desempenho, a qualidade de ovos, os parâmetros sanguíneos, o perfil bioquímico e o comportamento. Foram utilizadas 144 codornas japonesas, com aproximadamente 35 dias de idade, as quais foram distribuídas em delineamento inteiramente ao acaso, com quatro tratamentos e seis repetições cada. Foram fornecidas dietas com 0, 2, 4 e 6% de folhas desidratadas e moídas de moringa. A inclusão de moringa nas dietas reduziu quadraticamente o consumo até o nível de 1,2%, aumentou o peso dos ovos com comportamento quadrático até o nível de 3,8% e aumentou linearmente o peso da gema. A coloração da gema se intensificou com maiores inclusões de moringa. O perfil bioquímico sofreu alterações leves, mas não saiu dos padrões normais para codornas. A inclusão de 4% de moringa nas dietas aumentou os níveis de fosfatase alcalina. Já a inclusão de 6% reduziu níveis de colesterol e triglicérides. O comportamento das codornas não se alterou com a inclusão de moringa nas dietas. A inclusão de até 3,83% de *Moringa oleifera* na dieta de codornas japonesas aumenta a qualidade de ovos sem comprometer o desempenho, o perfil bioquímico, os parâmetros sanguíneos e o comportamento delas.

Palavras-chave: aditivos fitogênicos, antioxidantes, codornas de postura, plantas medicinais, qualidade de ovos

Recebido em 29 de setembro de 2020

Aceito em 19 de fevereiro de 2021

*Autor para correspondência (corresponding author)

E-mail: fariakita@gmail.com

INTRODUCTION

Compounds extracted from medicinal or herbal plants have beneficial effects due to their antioxidant action and improving nutrient digestibility. They stimulate enzyme secretion and benefit intestinal microflora, and consequently provides better performance, greater feed conversion and, higher egg production in laying birds, in addition to an increased internal egg quality (Boka *et al.*, 2014). We highlight the use of moringa (*Moringa oleifera*), which has several functions and applications in agriculture, medicine, and animal and human nutrition (Ndubuaku *et al.*, 2015). The fresh leaves have excellent nutritional qualities, as a good protein source, containing about 33.8% protein (Gallão *et al.*, 2006). It also contains vitamin A, B and C, and minerals such as iron, calcium, phosphorus, potassium, and zinc (Moyo *et al.*, 2011). Due to all of these characteristics, moringa has become a potential candidate as an alternative food in poultry feed mainly for egg production, in addition to acting on the productive aspects, moringa leaves can act by significantly reducing serum cholesterol, egg yolk cholesterol and pigmentation action, and can increase egg shelf life.

Research has revealed that moringa can be considered an important plant with multifunctional applications in livestock nutrition and products, dietary levels of 5% and 10% of moringa leaf produced positive results in increasing health, performance, and product quality (El-Hack *et al.*, 2018; Falowo *et al.*, 2018; Mahfuz and Piao, 2019). Despite the good nutritional characteristics that differentiate them from other forages, the ability of the birds to take advantage of their nutrients should be considered, as these animals have a limited ability to take advantage of fibrous feed (Donkor *et al.*, 2013). Also, anti-nutritional factors could limit moringa inclusion levels (El-Hack *et al.*, 2018) which indicates further research on the utilization of enzyme additives may be considered to improve nutrient digestibility (Falowo *et al.*, 2018). On the other hand, dietary levels of 15% and 20% of inclusion produced adverse effects on bird's health and performance.

The world's poultry chain is growing fast but laying hens may present reduced performance and egg quality, the increase in nutrient metabolism

during egg formation or weakened immune systems, directs dietary nutrients to health-maintenance (Moula *et al.*, 2019). Laying birds require high amounts of lipids during egg formation suggesting evaluation of alternative potential feed ingredients containing functional nutrients (Pál *et al.*, 2002). Quails are rustic animals compared to broilers and laying hens, in addition, despite undergoing genetic selection programs, they still fall short of laying hens. By passing through genetic selection, birds are known to have lost part of their diverse cecal microbiota, resulting in a probable low digestion of dietary fibers in this organ (Clench and Mathias, 1995). In this sense, due to less advanced genetic selection and rusticity, it is likely that quails still have a high digestibility of foods with a high fiber content, such as moringa, allowing an adequate use of nutrients. Based on the nutritional characteristics of moringa and the physiology of digestion of Japanese quails, the objective is to evaluate the effects of inclusion of moringa in quail diets on performance, egg quality, blood parameters, serum biochemical profile, and behavior of these animals.

MATERIALS AND METHODS

The trial was carried out in the quail shed located in the experimental warehouse of the Faculty of Agricultural Sciences of the Federal University of Grande Dourados (UFGD), in Dourados, MS, Brazil, in partnership with the University Center of Grande Dourados (UNIGRAN). The experimental protocol was performed with the approval of the Ethics Committee on Animal Use of the University Center of Grande Dourados (Unigran/Dourados) under protocol no. 052/18. The experimental period lasted 66 d, in which the first 10 d were used to adapt the birds to experimental diets and the remaining days were divided into two cycles of 28 d each. One hundred and forty-four Japanese quails with approximately 35-d-old (*Coturnix coturnix japonica*), weighing 148 ± 14 g, bought from a commercial company, were used in the trial.

The birds were housed in a conventional shed in parallel lots containing five floors. There were two cages per floor (25cm width, 35cm length, and 20cm height), corresponding to an area of 175cm²/housed bird. The lightening program used was 16 h of light per day. The cages were equipped with a nipple water trough and a chute

Moringa Oleifera...

trough, and excreta trimmers were positioned below the cages. Water and feed were provided *ad libitum* to the quails. The experimental design was completely randomized with four treatments and six replications. The treatments were inclusion of

0, 2, 4 and 6% of dried and ground moringa leaves in the diet, percentages calculated according to the recommendation of Rostagno *et al.* (2017) (Table 1).

Table 1. Approximate centesimal and calculated composition of the experimental Japanese quail diets with the inclusion of dried and ground moringa leaves (*Moringa oleifera*)

Ingredients	Level of <i>Moringa oleifera</i> (%)			
	0.0	2.0	4.0	6.0
Corn, ground grain 8.51%	48.69	49.00	50.00	47.10
Soybean bran 46%	34.07	33.76	33.32	35.65
Soy oil	2.000	2.000	1.340	2.,00
Salt	0.000	0.000	0.100	0.010
Calcium carbonate	5.24	5.24	5.24	5.24
Vitamin-Mineral complex ¹	4.00	4.00	4.00	4.00
Inert	6.00	4.00	2.00	0.00
<i>Moringa oleifera</i>	0.00	2.00	4.00	6.00
Total (kg)	100.00	100.00	100.00	100.00
	Calculated composition			
Metabolizable energy (kcal/kg)	2,800	2,776	2,800	2,800
Crude protein (%)	20.00	18.91	20.00	20.80
Crude fiber (%)	2.981	3.000	3.021	3.133
Total lysine (%)	1.076	1.074	1.071	1.135
Methionine + Total Cystine (%)	0.616	0.613	0.611	0.630
Total Methionine (%)	0.336	0.335	0.336	0.347
Total Threonine (%)	0.772	0.772	0.773	0.811
Total Tryptophan (%)	0.252	0.252	0.252	0.267
Calcium (%)	3.020	3.033	3.047	3.068
Total Phosphorus (%)	1.919	1.919	1.920	1.928
Sodium (%)	0.170	0.169	0.210	0.176

¹Inclusion of the complex per kg of feed: Calcium (mix) 1,600g/kg, Calcium (max) 400g/kg, Phosphorus (min) 148g/kg, Sodium (min) 80g/kg, Methionine (min) 86g/kg, Lysine (min) 72g/kg, Vitamin A (min) 500,000 IU/kg, Vitamin D3 (min) 100,000 IU/kg, Vitamin E (min) 1,248 IU/kg, Vitamin K3 (min) 80mg/kg, Vitamin B1 (min) 80mg/kg, Vitamin B2 (min) 250mg/kg, Vitamin B6 (min) 150mg/kg, Vitamin B 12 (min) 800 mcg/kg, Folic Acid (min) 25mg/kg, Pantothenic Acid (min) 500mg/kg, Biotin (min) 5mg/kg, Coline (min) 6,800mg/kg, Niacin (min) 1,248mg/kg, Copper (min) 500mg/kg, Iron (min) 2,720mg/kg, Iodine (min) 35mg/kg, Manganese (min) 3,748mg/kg, Selenium (min) 15mg/kg, Zinc (min) 2,000mg/kg, and Fluoride (max) 1,480mg/kg.

The moringa was collected at the UFGD experimental farm and only the leaves were used. They were dried at 65° C in an oven with forced ventilation. After drying, leaves were ground and could be mixed with the other ingredients of the diets. Moringa leaves bromatological composition is indicated in Table 2, the inclusion in diets considered its composition in the formulation of the diets. The quails were weighed at the beginning and at the end of the experimental period to determine bird weight (g) and weight gain (g). Feed intake (g/bird/day) was evaluated by weighing the feed offered and the leftovers at

the end of each stipulated period. Egg mass (g egg/bird/day), feed conversion by mass (FCm) and feed conversion by dozen (FCdz) were also evaluated. The values were obtained by dividing the averages of performance variables as described below:

$$FCdz = \frac{FI}{DZ} \quad FCm = \frac{FI}{EGGM}$$

EGGM = egg mass; DZ = dozens of eggs produced; FI: feed intake.

Table 2. Chemical composition of dried and ground moringa leaves *Moringa oleifera* used in experimental Japanese quail diets

Nutritive value	Dry leaf
Dry matter (%)	93
Crude Protein (%)	26.06
Gross Energy (kcal / kg)	3000
Ashes (%)	12
Ethereal extract (%)	8
Crude Fiber (%)	15
Calcium (%)	2.1
Copper (%)	0.0001
Iron (%)	0.027
Potassium (%)	1.3
Magnesium (%)	0.405
Phosphor (%)	0.31
Manganese (%)	0.008
Selenium (%)	0.0026
Zinc (%)	0.002
Molybdenum (%)	0.0005
Sodium (%)	0.1
Sulfur (%)	0.74
Arginine (%)	1.6
Histidine (%)	0.53
Isoleucine (%)	1.14
Leucine (%)	2.05
Lysine (%)	1.2
Methionine (%)	0.37
Phenylalanine (%)	1.4
Threonine (%)	1.08
Tryptophan	0.58
Valine (%)	1.4
B.C. Aspart (%)	1.67
B.C.glut (%)	2.47
Serine (%)	0.84
Glycine (%)	0.96
Alanine (%)	1.26
Proline (%)	1.23
Tyrosine (%)	0.91
Cysteine (%)	0.36

The average egg production per bird/day (%) was calculated by counting the eggs laid daily. The viability of the flocks was calculated using the number of deaths subtracted from the number of live birds. The values were converted into percentage at the end of the experimental period. The eggs of the last three days of each experimental period were collected and analyzed for egg quality in the Agricultural Product Analysis Laboratory (FCA/UFGD). The

evaluations consist of average egg weight, obtained through the average egg weight at the end of trial periods; specific gravity, obtained by immersing all eggs of each repetition in buckets with different saline solutions (NaCl), whose densities ranged from 1.050 to 1.100 (at intervals of 0.005, the density was measured using an INCOTERM-OM-5565 densimeter); yolk color on a light surface: the coloration was obtained by two methods: the first by the comparison with the Yolk Color Fan (DSM) using the color closest to the color palette, which varies from 1 to 15. The second method was the use of a Minolta colorimeter, which evaluates the parameters L* (luminosity), a* (red) and b* (yellow). Three different and equidistant surface readings were taken. The Chroma was obtained by the relation between the values of a* and b*, where the real color of the object is obtained, according to Harder *et al.* (2010). The Haugh unit correlates egg weight with albumen height and egg quality. The higher the Uh value, the better the egg quality, as expressed by the following formula:

$$U_h = 100 \log \left\{ H - \frac{\sqrt{G(30W^{0.37} - 100)}}{100} + 1.9 \right\}$$

H = height of dense albumen (millimeters); g = gravitational constant = 32; W = egg weight in grams (g).

The egg yolks were weighed, and the albumen weights were estimated by egg weight difference minus egg yolk weight plus shell weight, which was obtained after washing the shell and air drying it for 72 h. The albumen, yolk and shell percentage were obtained by dividing the weights of the respective components by the average egg weight, and the result was multiplied by 100. The shell weight and thickness were recorded after drying in a forced circulation oven for 24 h. The albumen height and bud height were measured using the Starret 150mm digital caliper by measuring the albumen and the yolk. The aggressive behavior (riding and pecking) and the non-aggressive behavior were analyzed (eating, drinking, scratching, and idleness). The procedure was performed by instant focal sampling by direct and continuous observation of a single bird from each cage randomly for five minutes in each cage. This procedure was performed during the final week of experimental period throughout the entire

light period (7:00 to 18:00 h). The observations of aggressiveness were made according to the methodology described by Savory *et al.* (1999).

The intensity of body injuries was evaluated according to the presence or absence of lesions in the following regions: back, tail, wings, and head in a single bird of each replication. To assess tonic immobility, the bird was quickly turned over and placed in a supine position on a flat surface. Then, pressure was exerted on its chest for three seconds. To be considered in a state of tonic immobility, the bird should remain immobile for at least ten seconds, according to Heiblum *et al.* (1998).

At the end of the experimental period, one quail of each replication was randomly chosen for biochemical and blood analysis. Blood samples were collected from the jugular vein of each slaughtered bird and immediately centrifuged for serum separation, after which they were frozen at -20° C until analysis. Biochemical and blood count analyses were performed. Biochemical tests were processed by spectrometry (BioPlus 200), as indicated by the manufacturer of the commercial kits (GoldAnalisa®). For biochemical analyses (cholesterol, triglycerides, creatinine, phosphatase, lactate, and glucose), 3 mL of blood samples were collected from the jugular vein without anticoagulants. Blood samples were kept in Eppendorf tubes for one hour at room temperature and centrifuged at 3,500 rpm and 4°C for 15 minutes for serum separation. The serum was frozen in a freezer at -70°C until analysis. The samples were prepared and analyzed according to the manufacturer's specifications. Three readings in birds/treatment were taken in a spectrophotometer (Beckman Coulter, DU-800) using a 500-nm wavelength. For blood count analysis (VG and PPT), 2 ml of jugular vein blood were collected in a heparinized syringe immediately after collection using a portable clinical analyzer (i-STAT®Co. - Abbott Laboratories - USA, Cg8+® cartridge).

The means of performance data, egg quality and biochemical profile obtained in the two evaluation periods were calculated. The data were tested for statistical assumptions of residue normality and variance homogeneity using the Shapiro-Wilk and Levene tests, respectively. Means that met these assumptions underwent analysis of variance at 5% probability using the SAS statistical package

(version 9.3) (Statistical..., 2012). Subsequently, the effects of moringa inclusion were estimated by analyzing the variables via orthogonal polynomials to obtain regression equations. The means of blood and biochemical parameters were compared between treatments with the Tukey test. Behavior and injury data were evaluated by non-parametric statistical analyses using the Chi-square test and the SAS statistical package (version 9.3) (Statistical..., (2012), considering a significance level of 5%. The level of significance for all variables analyzed was 5%.

RESULTS AND DISCUSSION

The inclusion of *Moringa oleifera* in the diets of Japanese quails does not influence the performance variables, except for feed intake, there was a quadratic reduction up to the level of 1.20% of moringa in the diet (Table 3). The main factor that may have caused the reduction of feed intake is the feed's crude fiber content, conferred by the increased inclusion of plant extract in the diet. By increasing moringa levels in the diet, the fibrous fraction also increased. This increase in fiber leads to an increase in the digestion passage rate, with a consequent reduction in nutrient absorption. In addition, it may impair nutrient use, as fiber acts as a physical barrier preventing endogenous enzymes from gaining access to the internal contents of plant cells, reducing nutrient digestion and absorption processes (Braz *et al.*, 2011).

Fiber-rich diets have lower gross energy metabolization coefficients, i.e., a lower energy use due to a lower fiber digestibility in the birds' digestive tract (Macambira *et al.*, 2018). It is noteworthy that in the present study, even with a lower intake, there was no impairment in feed conversion of birds, and conversion values were similar for all treatments. Elkloub *et al.* (2015) analyzed different levels of moringa inclusion (0, 2.0, 4.0 and 6.0%) in Japanese quail feed and found that the birds with the plant inclusion in diets had a better performance compared to the control group. Quails that received 2% moringa had a greater weight gain, a lower feed intake, and better feed conversion. Olugbemi *et al.* (2010) also observed the absence of negative effects on the performance of commercial laying hens fed on 0, 5 and 10% of moringa leaf meal as a substitute for soybean meal.

Table 3. Performance of Japanese quails fed with different levels of inclusion of dried and ground moringa leaves (*Moringa oleifera*) in diets

Variable	Level of <i>Moringa oleifera</i>				MSE	Regression	
	0	2	4	6		Linear	Quad.
AWG (g)	-0.134	-0.112	-0.164	-0.134	0.0710	0.8437	0.9459
Intake (g/bird/day)	51.56	50.01	51.23	46.73	0.5347	0.0004	0.0417
Laying rate (%)	73.67	73.67	72.04	72.99	0.5654	0.4303	0.6446
FC/dz (g/dz)	29.97	28.25	30.74	27.82	0.3722	0.3041	0.3602
Egg mass (g/bird/day)	18.02	18.24	18.95	18.20	0.2041	0.4690	0.2244
FC/egg mass (g/g)	2.27	2.31	2.12	2.38	0.036	0.5712	0.0620
Variable	Regression equation				R ²	Effect	Level
Intake (g/bird/day)	Y=51.1393+0.4436X-0.1846X ²				0.5064	Quad.	1.20

AWG: Average weight gain; FC/dz (g/dz): feed conversion per dozen eggs produced; FC/egg mass (g/g): Feed conversion per egg mass produced. Quad: quadratic effect. Regression: linear and quadratic models. R²: coefficient of determination

In moringa, there are also compounds that can act as antinutritional factors and impair performance, interfering with protein and amino acid digestibility, vitamin and mineral availability, as well as energy metabolism. By examining the nutritional composition and the antinutritional factors of different parts of the *Moringa oleifera* plant, Macambira *et al.* (2018) found low levels of tannins, saponins, phytates, oxalates, and protease inhibitors. These compounds reduce protein digestibility as they form saponin-protein complexes and cause damage to the intestinal mucosa by interacting with the lipid bilayer, facilitating the absorption of toxic substances. Protease inhibitors reduce protein digestion of food (Stenico *et al.*, 2017), providing a decreased weight gain and animal growth.

Regarding shell weight, albumen weight, albumen height, shell thickness, bud percentage, albumen percentage, shell percentage, and Haugh unit, no differences were found with the inclusion of moringa (Table 4). The specific gravity, egg weight, yolk weight, yolk height, albumen pH, yolk pH, yolk luminosity (L*), yolk red color (a*), yolk yellow color (b*), yolk color shade, and yolk Chroma, were affected by the inclusion of moringa in the diet (Table 5). Regarding specific gravity, there was a decreasing linear effect as the moringa inclusion levels increased. According to Freitas *et al.* (2011), the high specific gravity

results from a better shell quality because there is loss of water in the egg after laying as a result of evaporation, which causes a progressive increase in the air cell and consequently a decrease in egg specific gravity (Quadros *et al.*, 2011).

The egg weight variable showed a negative quadratic effect as the moringa levels increased, reaching a higher weight, the maximum egg weight was obtained with 3.83% of inclusion. The yolk weight had a linear increasing effect proportional to the increase of moringa inclusion in the diets. For yolk height, there was a positive quadratic effect of moringa inclusion in the diets. These results evidence positive effects of the inclusion of moringa in the diets, even though there is no remuneration related to egg size in marketing eggs. The albumen pH showed a decreasing linear effect for moringa inclusion levels, unlike yolk pH (negative quadratic effect). The lowest pH was achieved with a 5.40% moringa inclusion. According to Siebel (2005), the normal albumen pH should be around 7.9 and egg yolk should be 6.2 in freshly laid eggs. The average results obtained with the inclusion of moringa in the diets showed a pH above the desired value. The pH of eggs increases according to storage days. In the present study, this increase in pH observed in all tested samples is probably related to the analysis method, since the eggs were analyzed about 48 after oviposition.

Table 4. Quality determination variables of Japanese quail eggs with inclusion of different levels of dried and ground moringa leaves (*Moringa oleifera*) in the diets

Variable	Level of <i>Moringa oleifera</i>				MSE	P-value	
	0	2	4	6		Linear	Quad.
Specific gravity	1,082.07	1,082.60	1,080.93	1,076.87	0.6440	0.0018	0.0558
Egg weight	11.44	11.58	12.27	11.70	0.0772	0.0166	0.0102
Yolk weight	3.48	3.39	3.83	3.58	0.0477	0.0170	0.2206
Shell weight	0.95	0.97	1.00	0.99	0.0125	0.2125	0.5628
Albumen weight	7.01	7.22	7.44	7.13	0.0675	0.3170	0.0555
Yolk height	11.89	11.65	11.60	11.87	0.0644	0.8135	0.0353
Albumen height	5.08	5.14	5.32	5.27	0.1005	0.3068	0.7334
Shell thickness	0.298	0.284	0.288	0.290	0.0029	0.4401	0.1762
Yolk percentage	30.43	29.36	31.19	30.68	0.2964	0.3030	0.6166
Album percentage	61.22	62.26	60.65	60.86	0.3509	0.3775	0.5403
Shell percentage	8.35	8.38	8.16	8.45	0.1112	0.9426	0.5625
Haugh unit	92.59	92.66	93.20	93.35	0.5004	0.4181	0.9620
Albumen pH	8.80	8.58	8.51	8.18	0.0651	<0.0001	0.5487
Yolk pH	6.49	6.50	6.73	6.63	0.0465	0.0448	0.4527
L*	56.29	54.62	53.26	51.43	0.2831	<0.0001	0.8029
Yolk red color (a*)	-4.28	-1.91	-0.57	1.27	0.2777	<0.0001	0.1311
Yolk yellow color (b*)	36.65	43.37	43.21	44.63	0.5335	<0.0001	<0.0001
Color tone	-1.45	-1.42	-0.61	1.54	0.1679	<0.0001	<0.0001
Yolk Chroma	35.92	43.43	43.23	44.65	0.5203	<0.0001	<0.0001

Quad.: Quadratic effect

Table 5. Regression equations, coefficient of determination, and maximum and minimum levels obtained for Japanese quail egg quality variables with different levels of inclusion dried and ground moringa leaves of (*Moringa oleifera*) in the diet of Japanese quails

Variable	Regression equation	R ²	Level
Specific gravity	Y=1,083.2067 – 0.8633X	0.1523	-
Egg weight	Y=11.3463+0.3410X-0.0445X ²	0.1680	3.83
Yolk weight	Y=3.4581 + 0.0372X	0.6705	-
Yolk height	Y=11.8984 – 0.1920X+0.0233X ²	0.5686	4.12
Albumen pH	Y=8.8042 – 0.0957X	0.1832	-
Yolk pH	Y=6.4665 + 0.0724X-0.0067X ²	0.3467	5.40
Yolk brightness (L*)	Y=56.2874 – 0.7961X	0.6699	-
Yolk red color (a*)	Y=-4.0752 + 0.8997X	0.8896	-
Yolk yellow color (b*)	Y=37.6975 + 1.3390X	0.5339	-
Yolk color tone	Y=-1.4202-0.3099X + 0.1331X ²	0.8894	1.16
Yolk Chroma	Y=36.3885+3.5798X – 0.3800X ²	0.6734	4.71

Regarding yolk luminosity (L*), there was a linear decreasing effect related to levels of moringa in the diet. Regarding yolk red color (a*) and yolk yellow color (b*), there was a linear increasing effect as the inclusion of moringa increased. The yolk is rich in pigments, with carotenoids and riboflavin constituting 0.02% of the egg dry weight. They are arranged in concentric rings that vary in color depending on the laying bird's diet. The color changes with pigments present in corn or in synthetic substances added to the feed. The yellowish color of the yolk is mainly due to the presence of riboflavin, xanthophylls and β-

carotene. Alternative plant foods, such as moringa, have different pigment compositions that may change yolk color. In the present study, the addition of moringa to diets favored a more intense yolk color, a factor of great importance to the market, as consumers prefer larger eggs and darker yolks (Seeman, 2000; Preisinger, 2018). Mabusela *et al.* (2018), using a whole *Moringa oleifera* seed cake in quail diet and inclusion levels of 0, 1, 3 and 5%, also observed an improvement in yolk color according to the increased inclusion of this byproduct, which corroborates with the findings of this study.

According to the evaluated blood parameters (Table 6), the globular volume (GV) of quails

submitted to treatments with inclusion of 0, 2, 4 and 6% of moringa did not differ.

Table 6. Blood and biochemical parameters of Japanese quail with different levels of inclusion of dried and ground moringa leaves (*Moringa oleifera*) in diets

Variable	Level of <i>Moringa oleifera</i>				MSE	P-value
	0	2	4	6		
GV (%)	26.99	20.79	29.41	27.79	0.470	0.076
TPP (g/dL)	6.477b	4.272c	7.012a	6.647b	0.434	0.025
Creatinine (mg/dL)	0.46a	0.336b	0.208d	0.269c	0.036	0.011
Phosphatase (IU)	428.5d	793.4b	1,340.25a	528.7c	0.212	0.029
Cholesterol (mg/dL)	90.87d	151.3b	183.28a	108.4c	0.341	0.043
Triglycerides (mg/dL)	341.7c	569.7b	658.97a	334.3d	0.473	0.003
Lactate (mg/dL)	3.347	4.64	4.247	3.896	0.669	0.055
Glucose (mg/dL)	315.3	220.1	300.47	302.7	0.190	0.011

* Means followed by different lowercase letters in lines differ statistically by Tukey test at 5% probability. gV: globular volume, TPP: total plasma protein; MSE: mean standard error.

When comparing the values obtained for total plasma protein (TPP), the treatment with 4% of moringa showed a difference from the treatments with other inclusion levels. Age may influence the diagnostic interpretation of laboratory data in birds (Lumeij, 1997). Montesinos *et al.* (1997) mentioned that variations in biochemical values, according to age, already occur since the first months of life. Mata (2018) reported that quails submitted to the inclusion of moringa in their diet have a similar hematological profile as that of quails fed on commercial rations, attesting the moringa as an alternative to keep the quails in a healthy and satisfactory clinical state. The concentration of TPP in birds is lower than in mammals, ranging from 2.5 to 6.0g/dL (Thrall, 2004). Therefore, the TPP values found in this study for quails submitted to moringa treatments did not show results outside the normal range for the species. Whereas Lu *et al.*, 2016 founded higher plasma total protein levels with dietary 5% for moringa leaf meal supplementation, which is a good indicator of the liver's synthetic function, similar to the present findings.

Regarding creatinine, the inclusion of 4% of EM obtained the lowest value, whereas the control diet resulted in the highest value for this parameter. The values obtained in the present study ranged from 0.2 to 0.4 (mg/dL). Schosler *et al.* (2013) found values ranging from 0.10 to 0.50mg/dL, demonstrating that EM did not change renal function, keeping levels within normal range. Regarding alkaline phosphatase, the lowest value was found in the 0% treatment and the highest in the 4% treatment. Alkaline phosphatase (AP) acts

in various tissues, such as bones and intestines. In laying birds, plasma AP activity results mainly from a high osteoblastic activity at the pre-ovulation phase in chickens and at the time of eggshell formation (Campbell, 2015). Alkaline phosphatase increases the concentration of inorganic phosphate acting on eggshell formation (Minafra *et al.*, 2008). A high alkaline phosphatase concentration in birds fed on moringa may indicate a positive influence on eggshell formation metabolism. When relating the egg and shell weight results obtained in the present study, there was an increase in weight for these variables when birds were fed at levels similar as 4%, which may indicate a positive effect of moringa on alkaline phosphatase metabolism.

Regarding cholesterol and triglycerides, the diet with 6% moringa inclusion was lower when compared to the other treatments. Cholesterol results in quails submitted to diets without the inclusion of moringa (0%) were below normal values (100 to 250mg/dL) (Silva *et al.*, 2012; Campbell, 2015). The reduced cholesterol levels of birds fed on high levels of moringa in diets may be related to the characteristic of some phytogetic additives that have lipophilic properties and phenolic compounds that may change the enzymatic activity and bile salts of birds, causing a greater digestibility and transport of lipids and reducing serum cholesterol (Attia *et al.*, 2003, Manafi, 2015). *Moringa oleifera* exerted no influence on the aggressive and non-aggressive behavior of Japanese quails (Table 7). Adult quails increase interaction (aggressive or non-

aggressive) as a result of regrouping with the aim of restoring the hierarchy of the group formed (Guzmán *et al.*, 2013). This phenomenon occurs mainly within the first 24 hours after exposure of birds to such social stress (Tenório *et al.*, 2017) or in the first week (Guzmán *et al.*, 2013). Therefore,

the greater expression of pecking and riding behaviors may be the result of the adaptation period of these birds until they reestablish the group hierarchy. After this period, there is a decrease in these behaviors, reflecting the absence of differences in the observation periods.

Table 7. Behavior and score of Japanese quail wounds with different levels of inclusion of dried and ground moringa leaves (*Moringa oleifera*) in diets

Behavior	Level of <i>Moringa oleifera</i> (%)				P-value*
	0	2	4	6	
Aggressive	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.7280
Pecking	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.2215
Mounting	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.4578
Not aggressive	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.5712
Eating	30.6% (15/49)	20.4% (10/49)	28.6% (14/49)	20.4% (10/49)	0.2754
Drinking	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.7626
Scratching	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.9908
Idleness	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.1503
TIT	30.0% (15/50)	20.0% (10/50)	30.0% (15/50)	20.0% (10/50)	0.6154
Injury score					
0	10% (10/100)	5% (5/100)	15% (15/100)	10% (10/100)	
1	10% (10/100)	16% (16/100)	2% (2/100)	5% (5/100)	0.0031
2	5% (5/100)	4% (4/100)	7% (7/100)	10% (10/100)	
3	0% (0/100)	0% (0/100)	1% (1/100)	0% (0/100)	

* Chi-square at 5% probability. TIT tonic immobility time.

Tonic immobility time (TIT) also showed no significant effects. Similar results were also found by Marques *et al.* (2010). This behavior is the last anti-predatory defense response of some species. It is characterized by pretending to be dead to create an opportunity to escape by inducing relaxation of the predator's attention (Brunelli *et al.*, 2018). Regarding injury scores, there was an effect of the inclusion of moringa in the diets, and the inclusion of 2% in the diets caused a high frequency of minor injuries. The inclusion of moringa was expected to be capable of producing anxiolytic effects in birds, but just as Gravena *et al.* (2009) pointed out using valerian, the inclusion of moringa in diets was not able to exert sedative or anxiolytic effects.

Marques *et al.* (2010), using chamomile as a phytotherapeutic additive for quails at the laying phase, did not observe a sedative or anxiolytic effect, showing no significant reduction of bodily injuries. Thus, the results of the present study were similar to the literature, moringa, as chamomile and valerian, has no calming effects on birds. The lesions resulting from the inclusion of moringa were mild and were only higher at the

2% level of inclusion. At the other levels, they were similar as the control diet, which may indicate that no deleterious effects of inclusion of these compounds to diets are noted. Despite positive results founded in the present study on the potential use of *Moringa oleifera* in quail diets to increase egg quality, without compromising performance, biochemical profile, blood parameters, and bird behavior the possibility of its use in quail diets is evident, however it is known that some challenges are still present, such as the presence of antinutritional factors (Falowo *et al.*, 2018), which still requires studies to further improve moringa dietary benefits.

CONCLUSION

Up to 3.83% of *Moringa oleifera* can be included in Japanese quail diet demonstrating general benefits to quail production, improving egg quality without compromising performance, biochemical profile, blood parameters, and bird behavior. Higher levels of *Moringa oleifera* inclusion also can bring benefits, however they showed occasional improvements in the evaluated variables.

REFERENCES

- ATTIA, Y.A.; TAG EL-DIN, A.E.; ZEWEIL, H.S. *et al.* Nutritional evaluation of nigella seed meal and the effect of microbial phytase and amino acids supplementations on its feeding value for Japanese quail. *Egypt. J. Nutr. Feeds*, v.6, p.201-217, 2003.
- BOKA, J.; MAHDAVI, A.H.; SAMIE, A.H. *et al.* Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal *Escherichia coli* colonization and jejunal morphology in laying hens. *J. Anim. Physiol. Anim. Nutr.*, v.98, p.373-383, 2014.
- BRAZ, N.; FREITAS, E.R.; BEZERRA, R.M. *et al.* Fibra na ração de crescimento e seus efeitos no desempenho de poedeiras nas fases de crescimento e postura. *Rev. Bras. Zootec.*, v.40, p.2744-2753, 2011.
- BRUNELLI, P.F.; ROMANIA, H.F.; CULTRI, G.R.S. *et al.* Bem-estar e qualidade de ovos de codornas alimentadas com extratos de camomila e passiflora. *Bol. Inst. Anim.*, v.75, p.1-11, 2018.
- CAMPBELL, T.W. *Hematologia e bioquímica clínica veterinária*. São Paulo: Roca, 2015. 678p.
- CLENCH, M.H.; MATHIAS, J.R. The avian cecum: a review. *Wilson Bull.*, v.107, p.93-121, 1995.
- DONKOR, A.M.; GLOVER, R.L.K.; ADDAE, D. *et al.* Estimating the nutritional value of the leaves of moringa oleifera on poultry. *Food. Nutr. Sci.*, v.4, p.1077-1083, 2013.
- EL-HACK, A.; MOHAMED, E.; ALAGAWANY, M. *et al.* Effect of forage moringa oleifera L.(moringa) on animal health and nutrition and its beneficial applications in soil, plants and water purification. *Agriculture*, v.8, p.145, 2018.
- ELKLOUB, K.; MOUSTAFA, M.E.; GHAZALAH, A.A. *et al.* Effect of dietary nanosilver on broiler performance. *Int. J. Poult. Sci.*, v. 14, p. 177-182, 2015.
- FALOWO, A.B.; MUKUMBO, F.E.; IDAMOKORO, E.M. *et al.* Multi-functional application of Moringa oleifera Lam. in nutrition and animal food products: a review. *Food Res. Int.*, v.106, p.317-334, 2018.
- FREITAS, L.W.; PAZ, I.C.D.L.A.; GARCIA, R.G. *et al.* Aspectos qualitativos de ovos comerciais submetidos a diferentes condições de armazenamento. *Agrarian*, v.4, p.66-72, 2011.
- GALLÃO, M.I.; DAMASCENO, L.F.; BRITO, E.S. Avaliação química e estrutural da semente de moringa. *Rev. Ciênc. Agron.*, v.37, p.6-109, 2006.
- GRAVENA, R.A.; MARQUES, R.H.; SILVA, J.D.T. *et al.* Uso da Valeriana officinalis em dietas de codornas japonesas na fase de postura. *Biotemas*, v.22, p.185-191, 2009.
- GUZMÁN, D.A.; PELLEGRINI, S.; KEMBRO, J.M. *et al.* Social interaction of juvenile Japanese quail classified by their permanence in proximity to a high or low density of conspecifics. *Poult. Sci.*, v.92, p.2567-2575, 2013.
- HARDER, M.N.C.; SPADA, F.P.; SAVINO, V.J.M. *et al.* Coloração de cortes cozidos de frangos alimentados com urucum. *Food Sci. Technol.*, v.30, p.507-509, 2010.
- HEIBLUM, R.; AIZENSTEIN, O.; GVARYAHU, G. *et al.* Tonic immobility and open field responses in domestic fowl chicks during the first week of life. *Appl. Anim. Behav. Sci.*, v.60, p.347-357, 1998.
- LU, W.; WANG, J.; ZHANG, H.J.; WU, S.G.; QI, G.H. Evaluation of Moringa oleifera leaf in laying hens: Effects on laying performance, egg quality, plasma biochemistry and organ histopathological indices. *Ital. J. Anim. Sci.*, v.15, p.658-665, 2016.
- LUMEIJ, J.T. Avian clinical biochemistry. In: KANEKO, J.J. *Clinical biochemistry of domestic animals*. San Diego: Academic, 1997. p.857-883.
- MABUSELA, S.P.; NKUKWANA, T.T.; MOKOMA, M. *et al.* Layer performance, fatty acid profile and the quality of eggs from hens supplemented with Moringa oleifera whole seed meal. *S. Afr. J. Anim. Sci.*, v.48, p.234-243, 2018.
- MACAMBIRA, G.M.; RABELLO, C.B.V.; NAVARRO, M.I.V. *et al.* Caracterização nutricional das folhas de Moringa oleifera (MOL) para frangos de corte. *Arq. Bras. Med. Vet. Zootec.*, v.70, p.570-578, 2018.
- MAHFUZ, S.; PIAO, X.S. Application of Moringa (*Moringa oleifera*) as natural feed supplement in poultry diets. *Animals*, v.9, p.431, 2019.

- MANAFI, M. Comparison study of a natural non-antibiotic growth promoter and a commercial probiotic on growth performance, immune response and biochemical parameters of broiler chicks. *J. Poult. Sci.*, v.52 p.274-281, 2015.
- MARQUES, R.H.; GRAVENA, R.A.; SILVA, J.D.T. *et al.* Inclusão da camomila no desempenho, comportamento e estresse em codornas durante a fase de recria. *Cienc. Rural*, v.2, v.40, p.415-420, 2010.
- MATA, Y.D.D. *Análise hematológica de codornas europeias alimentadas com diferentes níveis de moringa (moringa oleifera)*. 2018, 40f. Dissertação (Mestrado) - Universidade Federal Rural do Semi-Árido, Mossóro, RN.
- MINAFRA, C.S.; MORAES, G.H.K.; RODRIGUES, A.C.P. *et al.* Perfil bioquímico e nutricional do ácido glutâmico e da vitamina K no soro e no fígado de frangos de corte de 1 a 21 dias de idade. *Rev. Bras. Zootec.*, v.37, p.1973-1977, 2008.
- MONTESINOS, A.; SAINZ, A.; PABLOS, M.V. *et al.* Hematological and plasma biochemical reference intervals in young white storks. *J. Wildl. Dis.*, v.33, p.405-412, 1997.
- MOULA, N.; SADOUDI, A.; TOUAZI, L. *et al.* Effects of stinging nettle (*Urtica dioica*) powder on laying performance, egg quality, and serum biochemical parameters of Japanese quails. *Anim. Nut.*, v.5, p. 410-415, 2019.
- MOYO, B.; MASIKA, P.J.; HUGO, A. *et al.* Nutritional characterization of moringa (*Moringa oleifera* Lam.) leaves. *Afr. J. Biotechnol.*, v.10, p.12925-12933, 2011.
- NDUBUAKU, U.M.; UCHENNA, N.V.; BAIYER, K.P. *et al.* Anti-nutrient, vitamin and other phytochemical compositions of old and succulent moringa (*Moringa oleifera* Lam) leaves as influenced by poultry manure application. *Afr. J. Biotechnol.*, v. 14, p. 2502-2509, 2015.
- OLUGBEMI, T.S.; MUTAYOBA, S.K.; LEKULE, F.P. Effect of Moringa (*Moringa oleifera*) inclusion in cassava based diets fed to broiler chickens. *Int. J. Poult. Sci.*, v.9, p.363-367, 2010.
- PAL, L.; GROSSMANN, R.; DUBLECZ, K. *et al.* Effects of glucagon and insulin on plasma glucose, triglyceride, and triglyceride-rich lipoprotein concentrations in laying hens fed diets containing different types of fats. *Poult. Sci.*, v.81, p.1694-1702, 2002.
- PREISINGER, R. Innovative layer genetics to handle global challenges in egg production. *Br. Poult. Sci.*, v.59, p.1-6, 2018.
- QUADROS, D.G.; JESUS, T.R.; KANEMATSU, C.H. *et al.* Qualidade de ovos de galinha comercializados em Barreiras, BA, estocados em diferentes condições de temperatura. *Rev. Acad. Cienc. Anim.*, v.9, p.363-369, 2011.
- ROSTAGNO, H.S.; ALBINO, L.F.T.; HANNAS, M.I. *et al.* *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais*. 4ed. Viçosa, MG: UFV Departamento de Zootecnia, MG, 2017. 488p.
- SAVORY, C.J.; MANN, J.S.; MACLEOD, M.G. Incidence of pecking damage in growing bantams in relation to food form, group size, stocking density, dietary tryptophan concentration and dietary protein source. *Br. Poult. Sci.*, v.40, p.579-584, 1999.
- SCHOSSLER, J.E.W.; SERAFINI, G.M.C.; LUCAS, S.S. Valores laboratoriais e aspectos histológicos de codornas domésticas (*Coturnix coturnix japonica*). *Rev. Cien. Elet. Med. Vet.*, v.21, p.1-13, 2013.
- SEEMANN, M. Factors which influence pigmentation. *Lohmann Inf.*, v.24, p.20-24, 2000.
- SEIBEL, N.F. Transformações bioquímicas durante o processamento do ovo. In: SOUZA-SOARES, L.A.; SIEWERDT, F. *Aves e ovos*. Pelotas: UFPEL. 2005, p.77-90.
- SILVA, J.E.S.; MOURA, A.M.A.; NOGUEIRA, R.A. Efeito dos ácidos graxos essenciais sobre lipídemia e vascularização da membrana vitelina de codornas japonesas. *Arq. Bras. Med. Vet. Zoot.*, v. 64, p. 1603-1612, 2012.
- STATISTICAL analysis system. SAS PC Windows Version 9.2.0. Cary: SAS Institute Inc., 2012.

STENICO, M.E.; LORENZI, A.S.; SILVA, C.S. *et al.* Inibidores de proteases produzidos por cianobactérias: uma revisão. *Oecol. Aust.*, v.16, p.183-209, 2017.

TENÓRIO, K.I.; SGAVIOLI, S.; RORIZ, B.C. *et al.* Effect of chamomile extract on the welfare of laying Japanese quail. *Rev. Bras. Zootec.*, v.46, p.760-765, 2017.

THRALL, M.A. Laboratory evaluation of plasma and serum proteins. In: THRALL, M.A.; WEISER, G.A.; ROBIN, W. *Veterinary hematology and clinical chemistry*. Philadelphia: Lippincott Williams, 2004. p.401-412.