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Bee pollen improves productivity of laying Japanese quails

Pólen apícola melhora a produtividade de codornas Japonesas em postura

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SUMMARY

This study aimed to evaluate the effects of bee pollen (BP) on the performance of laying Japanese quails and egg quality. A total of 60 quails were used in a completely randomized experimental design with four treatments (0%, 0.5%, 1%, and 1.5% BP inclusion in diets) and five replicates. At the end of the study, productive performance and egg quality were evaluated; 160 eggs were maintained at different temperatures based on a completely randomized design and factorial arrangement (4 × 2), with four BP levels (cited above) and two storage temperatures (room temperature and refrigeration) for 14 days, totaling eight treatments with four replicates. BP levels did not influence (P> 0.05) feed conversion; however, daily feed intake, laying rate, egg mass, and weight and percentage of albumen increased. Yolk percentage decreased due to the augmentation of BP in diets. There was no effect (P> 0.05) of interaction of storage temperature × BP levels on egg quality, except with regard to the Haugh unit. BP supplementation resulted in higher egg weight and index of yolk and albumen. Refrigerated eggs had better internal quality than normal eggs. BP inclusion in diets of Japanese quails improved bird performance and the quality of fresh and stored eggs.
Keywords: animal nutrition, additive, bee product, egg production, poultry feeding

RESUMO

Este estudo teve como objetivo avaliar o efeito do pólen apícola sobre o desempenho de codornas Japonesas em postura e sobre a qualidade dos ovos. Foram usadas 160 codornas em delineamento completamente casualizado com quatro tratamentos (0%, 0,5%, 1%, e 1,5% de pólen apícola nas dietas) e cinco repetições. Ao fim do estudo, o desempenho e a qualidade dos ovos foram avaliados. Posteriormente, 160 ovos foram mantidos em temperaturas diferentes em delineamento completamente casualizado e arranjo fatorial 4 × 2, com quatro níveis de pólen apícola (citados acima) e duas temperaturas de

armazenamento (ambiente e refrigeração) por 14 dias, totalizando oito tratamentos com cinco repetições. Os níveis de pólen apícola não influenciaram (P>0,05) a conversão alimentar, entretanto o consumo diário de ração, a taxa de postura, a massa de ovo e peso e porcentagem de albúmen aumentaram e a porcentagem de gema diminuiu com o aumento do pólen apícola nas dietas. Não houve efeito (P>0,05) da interação temperatura de armazenamento × níveis de pólen apícola sobre a qualidade dos ovos, exceto pela unidade Haugh. A suplementação com pólen apícola resultou em maior peso de ovo e de índices de gema e de albúmen. Ovos refrigerados tinham melhor qualidade interna. A inclusão de pólen apícola nas dietas de codornas Japonesas melhorou o desempenho e a qualidade de ovos frescos e armazenados.
Palavras-chave: aditivo, alimentação de aves, nutrição animal, produção de ovos, produto apícola.

INTRODUCTION

Bee pollen (BP) has therapeutic assets, such as antioxidant (Kocot et al., 2018), antibacterial (Karadal et al., 2018), and immunomodulatory properties (Nascimento & Luz, 2018), as well as a high nutritive value, with 25.66% carbohydrates, 2.22–4.03% ash, 2.28–10.81% fat, and 19.59–37.63% protein. In addition, BP has vitamins and minerals, such as K, Ca, and Mg, and is rich in essential fatty acids, and amino acids (histidine, leucine isoleucine, tryptophan, valine, and lysine) (Rebelo et al., 2016; Negrão & Orsi, 2018; Taha et al., 2019).

Based on these beneficial properties, BP may improve the use and absorption of nutrients leading to a better productive performance in animals, such as better weight gain, feed intake and feed conversion in quails (Babaei et al., 2016; Desoky & Kamel, 2018), laying hens (Demir & Kaya, 2020), and broiler chickens (Hosseini et al., 2016).

The internal quality of the egg can be affected by nutrition and the relative humidity and temperature at which the eggs are stored, *inter alia* (Figueiredo et al., 2013). According to Feddern et al. (2017), from pasture to consumer table, physicochemical changes occur in yolk and/or albumen that may modify flavor, freshness, and palatability in eggs.

Consumer demand for natural products that can prevent the deterioration of animal-origin food has increased (Mitterer-Daltoé et al., 2020). Lipid oxidation caused by free radicals is one of the main mechanisms of quality deterioration (Yilmaz et al., 2017). Several studies have been carried out to evaluate the effect of natural antioxidants in laying diets (Cimrin et al., 2019) to prevent egg deterioration. BP may have a beneficial effect on the quality of eggs because it contains antioxidant substances, such as flavonoids, carotenoids, and phenolic compounds (Feas et al., 2012; Karadal et al., 2018).

The effects of storage temperature on egg quality are well known and the effects of antioxidant compounds on the quality of eggs stored for different lengths of time and/or temperatures have been studied (Baylan et al., 2011; Al-Harthi, 2014; Cimrin et al., 2019). However, research regarding the use of BP as an antioxidant for improving egg quality is scarce.

This present research was carried out to evaluate the effects of BP on the productive performance of laying Japanese quail and on the internal quality of fresh eggs or eggs stored under

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different temperatures up to 14 days after laying.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethics Committee on Animal Use (protocol number 01/12; April 19, 2012) of the Universidade de Rio Verde.
One hundred and sixty Japanese quail (*Coturnix japonica*), with an initial age of 50 days, were used for 84 days in a completely randomized design with four treatments and five replicates. Treatments consisted of BP inclusion in quail diets (0%, 0.5%, 1%, and 1.5% BP) (Table 1). Diets were formulated to meet the nutritional requirements of laying quail (Rostagno et al., 2011) and were provided *ad libitum*, twice a day. The BP used had a moisture content of 3.83%, crude protein content of 22.97%, gross energy content of 3953 kcal/kg, calcium content of 0.39%, phosphorus content of 0.99%, mineral ash content of 3.14%, fat content of 1.71%, and a pH of 4.68.
The light program was initiated on the fortieth day of age, with exposure of 14 h of light daily and a weekly increase of 30 min until reaching 17 h of daily light exposure, which was kept until the end of the experimental period.

Table 1. Composition of the experimental diets

	Bee pollen levels (%)			
Ingredients (kg)	**0.0**	**0.5**	**1.0**	**1.5**
Ground corn	49.83	49.83	49.83	49.83
Soybean meal	35.00	35.00	35.00	35.00
Soybean oil	3.88	3.88	3.88	3.88
Limestone	7.76	7.76	7.76	7.76
Dicalcium phosphate	1.13	1.13	1.13	1.13
Common salt	0.35	0.35	0.35	0.35
DL-methionine	0.05	0.05	0.05	0.05
Bee pollen	0.00	0.50	1.00	1.50
Caolin	1.50	1.00	0.50	0.00
Premix min/vit[1]	0.50	0.50	0.50	0.50
Calculated composition[2]				
Crude protein (%)	19.75	19.75	19.75	19.75
Metabolizable energy (kcal/kg)	2815	2815	2815	2815
Calcium (%)	3.09	3.09	3.09	3.09
Available phosphorus (%)	0.32	0.32	0.32	0.32
Sodium (%)	0.16	0.16	0.16	0.16
Total lysine (%)	1.10	1.10	1.10	1.10
Total methionine (%)	0.44	0.44	0.44	0.44
Total methionine + cystine (%)	0.78	0.78	0.78	0.78

[1]Each kg contain: 1800000 UI vit A, 500000 UI vit D_3, 2.000 UI vit E, 360 mg vit K_3, 2400 mcg vit B_{12}, 5000 mg niacin, 2000 mg pantothenic acid, 80 mg folic acid, 300 mg thiamine, 100 g choline, 1000 mg riboflavin, 300 mg pyridoxine, 8 mg biotin, 2000 mg Cu, 8000 mg Fe, 200 mg I, 15 g Mn, 60 mg Se, 10000 mg Zn, 20 g methionine, 6000 mg chloro hydroxyquinoline, 500 mg antioxidant.
[2]According to Rostagno *et al.* (2011).

Feed intake, laying rate, egg mass, feed conversion, and egg quality were evaluated. All the eggs laid in the three last days of the experimental period were

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used to determine egg weight and pH, yolk and albumen weight, percentage, quality related indices, and eggshell thickness. Yolk and albumen height and diameter were measured using a manual caliper, and the values were used to obtain their indices. Ten eggs of each replicate were used to determine the specific weight in saline solutions of a density of 1.050 to 1.100 g/cm^3.

Eggshells were washed and dried in air for later measurements (weight and thickness). Eggshell thickness was measured at three different points (on both poles and on the lateral part) with a digital caliper with 0.01 mm precision. The Haugh unit (HU) was obtained using the formula: $HU = 100 \times \log (H–1.7 \times P^{0.37} + 7.6)$, where H is the albumen height (mm) and P is the egg weight (g).

The results of productivity and egg quality were submitted to an analysis of variance and means were compared by the Tukey test at 5% probability using the software, SISVAR (Ferreira, 2011).

At the end of the experiment, 160 eggs were used for evaluating the effects of BP on the quality of eggs stored at room temperature or in the fridge for 14 days, in a completely randomized design and factorial arrangement (4 × 2), with four BP levels and two storage temperatures, ambient (29 °C ± 0.8 °C) and refrigeration (5 °C ± 0.6 °C), totaling eight treatments with four replicates. Egg weight, pH, yolk and albumen weight, percentage, and quality indices, and Haugh unit were evaluated as mentioned above. Data obtained with stored eggs were also submitted to an analysis of variance, and when the F test was significant for BP levels or BP × storing temperature, means were compared using the Tukey test at 5% probability using the software SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Feed conversion, kg/kg or kg/dozen eggs, was not affected ($P > 0.05$) by the treatments; however, daily feed intake, laying rate, and egg mass increased ($P < 0.05$) as BP levels increased in quail diets up to 1–1.5% (Table 2).

Table 2. Egg production performance of Japanese quail fed diets with bee pollen (BP)

	Bee pollen levels (%)					
Parameters	**0.0**	**0.5**	**1.0**	**1.5**	**SEM**	**p value**
Daily feed intake, g/day	27.01^b	26.86^b	29.44^{ab}	30.69^a	0.91	0.029
Feed conversion, kg/kg	3.72	3.32	2.93	3.15	0.25	0.206
Feed conversion, kg/dozen	0.49	0.44	0.42	0.42	0.03	0.228
Laying rate, %	66.39^b	72.18^b	85.19^a	87.74^a	3.09	0.001
Egg mass, g/day	7.36^c	8.17^{bc}	10.13^a	9.82^{ab}	0.54	0.010

SEM – standard error of the mean.
^{abc}Means followed by different letters in the row, are different by Tukey test at 5%.

Higher feed intake was reflected in better laying rate and egg mass. According to Peric et al. (2009), BP is an appetite stimulant, and this effect was also reported by Hosseini et al. (2016), who verified higher feed intake by broilers supplemented with BP, which resulted in higher nutrient ingestion. Babaei et al. (2016) noted that the addition of 5 g/kg BP was beneficial in improving Japanese quail performance. Canogullari et al. (2009) also supplemented Japanese quail with BP and did not observe effects on the productive performance, but the

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authors noted that feed intake increased with 1% and 2% BP inclusion in diets. These positive effects could be due to the nutritive value of BP, acting as a good source of fat, unsaturated fatty acids, proteins, essential amino acids, minerals, and carbohydrates (Abdelnour et al., 2019).

Treatment did not affect egg quality ($P > 0.05$), except for yolk percentage, which was reduced ($P \leq 0.003$), or albumen percentage, which was increased ($P \leq 0.006$), as BP levels increased in the diets (Table 3).

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Table 3. Characteristics of the eggs of Japanese quail fed diets with different inclusions of bee pollen (BP)

	Bee pollen levels (%)					
Parameters	**0.0**	**0.5**	**1.0**	**1.5**	**SEM**	**p value**
Egg						
Weight (g)	11.05	11.38	11.54	12.04	0.31	0.203
pH	7.47	7.45	7.55	7.42	0.05	0.278
Specific weight (g/cm^3)	1.061	1.063	1.065	1.066	0.001	0.186
Haugh unit	89.49	90.28	91.28	88.77	1.44	0.648
Yolk						
Weight (g)	4.00	3.80	3.80	3.20	0.23	0.130
Percentage (%)	36.7^{a}	33.37^{a}	32.61^{ab}	27.81^{b}	1.74	0.018
Index	0.508	0.517	0.494	0.513	0.013	0.676
Albumen						
Weight (g)	6.15^{b}	6.69^{b}	6.91^{ab}	7.87^{a}	0.35	0.022
Percentage (%)	55.54^{b}	58.80^{b}	59.81^{ab}	65.28^{a}	1.94	0.020
Index	0.146	0.102	0.100	0.098	0.026	0.514
Eggshell						
Weight (g)	0.85	0.88	0.87	0.83	0.04	0.694
Percentage (%)	7.69	7.82	7.58	6.92	0.42	0.457
Thickness (mm)	0.220	0.212	0.212	0.216	0.009	0.918

SEM – standard error of the mean.
[ab]Means followed by different letters in the row, are different by Tukey Test at 5%.

The yolk index is a measure of egg freshness and, as there was no difference in this parameter, it can be stated that BP inclusion did not affect yolk quality in fresh eggs. The reduction in yolk percentage and increase in albumen percentage occurred, possibly due to a higher protein deposition in the albumen. The need for amino acids for albumen synthesis is high, and any increase in this nutrient consumption and/or retention could improve the albumen amount in the egg, thereby decreasing yolk percentage, as demonstrated by Silva et al. (2010), who evaluated diets with 12%, 14%, 16%, and 18% crude protein for laying hens and noted higher protein consumption and higher albumen percentage when dietary protein content was increased.

In addition, BP contains important nutrients necessary for cellular differentiation, which results in trophic effects in the intestinal mucosa that increase the absorption surface (Fazayeli-Rad et al., 2015) and improve dietary nutrient use by the animal (Zeedan et al., 2017). Similar results were found by Demir and Kaya (2020). However, Desoky and Kamel (2018) observed improvements in the shell thickness and yolk index due to BP inclusion (0.2%) in diets of Japanese quail, and Arpášová et al. (2013) did not note differences in egg quality of laying hens fed 0.04% BP in their diets.

BP inclusion increased ($P \leq 0.02$) egg and albumen weight, egg pH, yolk and albumen indexes, and eggs stored under ambient temperature had a higher ($P \leq 0.02$) pH value, lower yolk, and albumen indexes. However, at 14 days, there was an effect ($P < 0.05$) of the BP × storing temperature interaction only on the Haugh unit value. Higher values were obtained with BP inclusion of 1–1.5% in diets (Table 4).

Egg freshness is a quality factor that can be influenced by the storage time and conditions (temperature and relative humidity), and by the egg age. The most common indicators of egg freshness are the height of the air space, albumen pH, aging rate, oxidation intensity of the yolk fats, and Haugh unit (Baylan et al., 2011). However, as in any other animal origin product, the egg starts to deteriorate immediately after egg laying. In our study, feed intake increased by 13.62% due to the inclusion of 1.5% BP; consequently, nutrient intake was also increased. One of the factors affecting egg weight is daily feed and nutrient intake, mainly protein intake. Egg weight increases with the increase in dietary protein ingestion (Lofti et al., 2018). According to Hegab and Hanafy (2019) and Yasin and Sultan (2020), heavier eggs are positively correlated with yolk and albumen indices and albumen weight.

Egg pH was higher when BP was included in the diets, probably because heavier eggs, tend to have a greater eggshell surface, and total pore count, leading to higher CO_2 losses and consequently, a higher egg pH. This effect was observed by El-Safty (2012) and Hegab and Hanafy (2019) in ostrich and Japanese quail eggs, respectively.

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Table 4. Characteristics of the eggs of Japanese quail fed diets with bee pollen (BP) stored under room temperature or refrigeration for 14 days

Treatments	Egg Weight (g)	Egg pH	Egg Haugh unit	Yolk Weight (g)	Yolk Percentage (%)	Yolk Index	Albumen Weight (g)	Albumen Percentage (%)	Albumen Index
				Effect of bee pollen levels (%)					
0.0	9.8[b]	7.4[b]	88.7[b]	3.4	33.8	0.32[b]	5.2[b]	52.9	0.09[b]
0.5	10.9[a]	7.8[a]	87.4[b]	3.5	31.7	0.34[b]	6.0[a]	55.0	0.09[b]
1.0	10.5[ab]	8.0[a]	91.6[a]	3.2	30.4	0.37[a]	6.0[a]	56.3	0.11[a]
1.5	11.0[a]	8.0[a]	89.1[ab]	3.6	31.4	0.35[ab]	6.0[a]	55.8	0.10[ab]
				Effect of storage temperature					
RT	10.5	7.9[a]	86.8[b]	3.5	31.9	0.27[b]	5.9	55.4	0.09[b]
UR	10.7	7.6[b]	91.6[a]	3.3	31.6	0.42[a]	5.8	54.5	0.12[a]
				Effect of the interaction bee pollen × storage temperature					
0.0 × RT	10.5	7.7	83.2[d]	3.6	33.8	0.24	5.5	53.5	0.08
0.5 × RT	10.9	7.9	85.9[cd]	3.6	32.1	0.27	6.0	55.0	0.08
1.0 × RT	10.5	8.1	89.8[b]	3.2	30.6	0.29	6.0	56.9	0.09
1.5 × RT	11.0	8.0	88.1[bc]	3.5	31.2	0.26	6.0	56.3	0.09
0.0 × UR	9.1	7.1	94.2[a]	3.0	33.2	0.40	4.8	52.2	0.12
0.5 × UR	10.9	7.7	88.9[bc]	3.4	31.3	0.41	6.0	55.1	0.09
1.0 × UR	10.4	7.9	93.4[a]	3.1	30.2	0.46	5.9	55.6	0.13
1.5 × UR	11.8	7.7	90.2[b]	3.7	31.6	0.43	6.5	55.2	0.11
SEM	0.3	0.1	0.7	0.1	0.9	0.01	0.2	0.9	0.04
				p values					
BP	0.032	0.013	0.003	0.223	0.097	0.023	0.011	0.938	0.028
ST	0.639	0.021	0.001	0.277	0.303	0.001	0.614	0.733	0.001
BP × ST	0.246	0.551	0.001	0.318	0.642	0.764	0.293	0.897	0.092

RT – room temperature, UR – under refrigeration, BP – bee pollen, and ST – storage temperature.
SEM – standard error of the mean.
[abc]Means followed by different letters in the row, are different by Tukey Test at 5%.

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Maintenance of the eggs at ambient temperature resulted in higher pH values, and yolk and albumen indices. All these deteriorations in the egg occurred due to ovalbumin degradation, which leads to water formation. This water migrates to the yolk, increasing its weight and percentage. This is one of the buffer system components in the albumen, and the dissociation forms water and CO_2. This CO_2 is lost through the eggshell pores, causing a pH increase (Eke et al., 2013). These reactions result in a decrease in internal egg quality, as measured by the yolk and albumen indices, as well as the Haugh unit of eggs stored at ambient temperature.

There was an interaction between BP and storage time and this is because BP has antioxidant activity (Karadal et al., 2018) due to the presence of several phenolic compounds in its composition, besides flavonoids and carotenoids (Feas et al., 2012). The Haugh unit is a function of the height of thick albumen and the weight of the egg. As BP inclusion in the diet improved the egg weight and albumen index, it was expected that the Haugh unit would also improve with the consequent improvement in egg internal quality.

BP inclusion at 1 and 1.5 ppm improved the Haugh unit of eggs stored at ambient temperature, because BP acts as an antioxidant (Kocot et al., 2018), inhibiting lipid oxidation in the yolk and ovalbumin degradation. These reactions would lead to water and CO_2 loss, reducing the egg weight and albumen height, with a consequent increase in pH and a decrease in Haugh unit values, which are internal quality measures of the egg.

Similar results were observed by other authors who worked with eggs stored under different temperatures and/or using additives with antioxidant activity in bird diets (Olobatoke & Mugueta, 2012; Arpásová et al., 2013; Al-Harthi, 2014; Lee et al., 2016; Feddern et al., 2017; Lana et al. 2017; Yilmaz et al., 2017).

CONCLUSION

The inclusion of BP in the diet of laying Japanese quails may improve their productive performance and the internal quality of fresh eggs while improving the Haugh unit in eggs stored at ambient temperature for 14 days. Storage of eggs under refrigeration improves egg pH, yolk, and albumen indices, resulting in better internal quality of the eggs.

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