JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

THE EFFECT OF BEE BREAD ON THE EGG QUALITY OF JAPANESE QUAILS DURING LAYING PERIOD

Emília Hanusová[1], Marta Oravcová[1], Cyril Hrnčár[2], Anton Hanus[1], Anna Kalafová[3], Marcela Capcarová[3]

Address(es): RNDr. Emília Hanusová, PhD.
[1]National Agricultural and Food Centre – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41Lužianky, Slovakia.
[2]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[3]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Science, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

*Corresponding author: emilia.hanusova@nppc.sk

https://doi.org/10.15414/jmbfs.4092

ARTICLE INFO

Received 15. 12. 2020
Revised 7. 9. 2021
Accepted 4. 10. 2021
Published 1. 12. 2021

Regular article

OPEN ACCESS

ABSTRACT

The aim of the study was to detect effect of supplementation bee bread in diet on Japanese quail's egg quality during laying. The quails were divided into four groups as follows: E1 with addition of 2 g, E2 with 4 g, E3 with 6 g bee bread per 1 kg of feed mixture and the control without additives (C). The groups were kept under the same conditions. The supplementation of BB into diets affected the laying parameters in favor of the experimental groups. The average egg weight was significantly ($P \leq 0.05$) affected by diet and by age. E2 had significantly lower egg weight than C and E3. The animals had significantly lower egg weight regardless of the experimental group at age of 16 weeks. The most significant differences between the C and E groups were in eggshell thickness. All experimental groups had a thinner eggshell than C. The average egg weight was significantly ($P \leq 0.05$) affected by diet and by age. The weight of the egg, yolk and albumen were significantly lower at the beginning of laying ($P<0.01$). We recorded significant differences between the control group and some experimental groups in the weight and width of the albumen; in the percentage of albumen and yolk between the control group and E2 ($P<0.05$). The control group had darker eggs (9.58°HLR) than experimental groups (from 8.89 to 9.34°HLR). We can conclude that the BB supplementation in diet had significant effect on selected parameters of egg quality of Japanese quails, mainly on the eggshell thickness.

Keywords: Japanese quail, Bee bread, egg, egg quality, laying

INTRODUCTION

In the field of animal production, the most growing sector include poultry farming. Japanese quail *(Coturnix japonica)* is the alternative poultry species, which can be used for egg production. Japanese quail is the smallest poultry species, which provides several advantages such as its resistance to numerous poultry diseases that affect chickens, its greater capacity to benefit from food, high reproduction proportions, and also low feed intake **(Santos *et al.*, 2011).** Recently, the Japanese quail is used in the world not only to produce eggs and meat, but also as a model animal for experiments (genetic, physiological, nutritional, ethological and technological), the conclusions of which also apply to other economically important avian species, especially hens and turkeys **(Genchev *et al.*, 2005, Hanusová *et al.*, 2018)**.
The egg quality is influenced by numerous factors such as the genotype of birds **(Genchev, 2012)**, selection **(Maiorano *et al.*, 2009)**, feeding **(Gardzielewska *et al.*, 2005)**, age **(Tserveni-Gousi and Yannakopoulos, 1986)** and stress **(Mota-Rojas *et al.*, 2007)**.
Al-Obaidi and Al-Shadeedi (2017) found no significant differences between chicken and quail egg in albumin content (11.76 *vs*. 11.80%) and egg yolk content (17.59 *vs*. 15.58%), respectively. They also found that quail egg cholesterol and LDL ($mg.g^{-1}$) levels were significantly lower than in the chicken egg. These findings imply that the quail egg is relatively healthier than the chicken egg especially for people suffering from cardiac problems.
The antibiotics growth promotors for poultry diets have been banned in the European Union in 2006. Therefore, studies on alternative products that could result in promotion of growth, improved feed utilization and maintains of gut health are taking place **(Zhang *et al.* 2005)**. For this reason, natural bee products are being widely investigated **(Babaei *et al.*, 2016; Haščík *et al.*, 2017, 2017; Pavelková *et al.*, 2020a, b)**. Honey and other bee products are also used in poultry diet for their anti-stress effects **(Tatli *et al.*, 2008, 2009; Ipek *et al.*, 2007)**.
The fashion for a healthy lifestyle leads to a situation where a number of people start taking care of their health. They search for the highest quality products, preferably with health benefits, rich in vitamins, valuable bioelements and nutrients. Biologically active substances of natural origin always arouse a great interest. This also applies to bee products because of their powerful healing properties. Bee products are multicomponent natural substances necessary for the proper course of basic life reactions **(Bobis *et al.*, 2010)**. These include the following: honey, pollen, bee bread, propolis, royal jelly, and bee venom. Bee products demonstrate a wide range of healing effects. They increase the level of ATP, thus neutralize an effect of many toxic agents, increase immunity of an organism, and improve the energy balance of tissues. They participate in many stages of protein metabolism.
Bee bread (ambrosia) is a unique product, which is very important not only for humans, but also for the bees. Bee bread (BB) is in fact fermented and naturally preserved pollen. Pollen is gathered by bees and mixed with its own digestive enzymes, carried in the hive and preserved with a tiny layer of honey and bee wax. It is a fermented bee product made from plant pollen, honey and bee saliva, which undergoes different chemical processes due to the action of specific enzymes, micro-organisms, moisture and temperature (35–36 °C) for 2 weeks **(Vásquez and Olofsson, 2009; Barajas *et al.*, 2012; Markiewicz-Zukowska *et al.*, 2013; Barene *et al.*, 2015; Fuenmayor *et al.*, 2014; Zuluaga *et al.*, 2015; Kieliszek *et al.*, 2018)**.
Bee bread is composed of balanced proteins containing all essential amino acids, the full spectrum of vitamins (C, B1, B2, E, H, P, nicotinic acid, folic acid), pantothenic acid, pigments and other biologically active compounds, like enzymes as saccharase, amylase, phosphatases, flavanoids, carotenoids, hormones **(Nagai *et al.*, 2005)**. According to the study by **Aliyazicioglu *et al.* (2005)**, bee bread is a product with high potential for use as a food supplement. Bee bread composition and bioactive proprieties have not been studied thoroughly until now and there are only few publications which present detailed studies of its chemical composition and proprieties. However, worldwide interest increases with highlighting chemical and therapeutic properties of bee bread.
Some studies indicate that fermentation process is necessary to modify the outer layer of the pollen known as exine, made of sporopollenin, a compound that provides chemical resistance to pollen and preserves the compounds which are within it, and is responsible for the limited capacity for absorbing nutrients and bioactive substances that are inside the pollen grain **(Atkin *et al.*, 2011** cited by **Zuluaga *et al.*, 2015)**. The bees can transform the bee pollen in bee bread by an

anaerobic fermentation process. Pollen transformation into bee bread occurs as a result of successive interventions of different enzymes, some species of microorganisms such as *Pseudomonas*, *Lactobacillus*, *Saccharomyces* that are naturally present in pollen, moisture and temperature (35–36º C) **(Barene *et al.*, 2015)**. During this fermentation, the wall of the pollen is disrupted and in this way the bee bread has a better bioavailability. Comparatively with bee pollen, beebread is better tolerated by the human organism and has a lower pH (3.8–4.3) duo to its content of lactic acid which offers a good stability during the storage at room temperature **(Berene *et al.*, 2015)**. Until now, there are only a few studies regarding the effects of bee bread in human health. The benefits of bee bread on the hepatic function and blood parameters in alcohol abuse patients suffering from chronic hepatitis was demonstrated in the study of **Čeksterytė *et al.* (2012)**. The results showed that bee bread helps to regulate the lipid metabolism and exerts a positive effect on the immune system of patients suffering from chronic arthritis and cardiovascular diseases and type 2 diabetes. The conclusion of this study was that bee bread used together with medicaments and Livosan supplement exerted a hepatoprotective effect and improved liver function.

Egg quality is factor which contributes for better economy price of fertile and table eggs **(Kocevski *et al*., 2011)**. Egg quality is usually commented in connection with consumers' requirements and is performed by groups of methods, which give a general characteristic of egg with intact eggshell (freshness, weight, size and shape, eggshell appearance) and the quality of egg parts (albumen, yolk and eggshell) **(Genchev, 2012)**. It has also been indicated that laying hen genotype (breed) and age have a significant impact on egg quality traits. It was demonstrated that egg weight depended on age **(Ferrante *et al.*, 2009; Simčič *et al.*, 2009; Haunshi *et al*., 2010; Samiullah *et al*., 2016)**. Egg shape was documented to depend on laying hen genotype **(Hanusová *et al.*, 2015; Ajmal *et al*., 2016)** and age **(Calik, 2011; Krawczyk, 2016)**. **Clerici *et al*. (2006), Zita *et al.* (2009) and Hanusová *et al.* (2015)** revealed that eggshell quality was dependent mostly on genetic traits while **Świątkiewicz and Koreleski (2008), Hincke (2012) and Küçükylmaz *et al.* (2012)** reported the influence of laying hen age on eggshell strength, thickness and density. The impact of hen age on albumen height and Haugh Unit (HU) value was reported by **Küçükylmaz *et al.* (2012), Hammershøj and Steefedt (2015)** and **Dudek and Rabsztyn (2011)**. Effect of genotype on egg quality characteristics of Japanese quail was analysed by **Hrnčár *et al.* (2014)**. The authors found significantly higher values for meat type in terms of all egg parameters ($P \leq 0.05$) when comparing with laying type. Eggshell integrity is important not only from economic point of view, but also regarding human health safety **(Genchev, 2012)**. The effect of bee products on egg quality in poultry followed several authors. Propolis and bee pollen were suggested as natural growth- promoter substitutions of antibiotics in poultry diets **(Kročko *et al*., 2012; Babaei *et al*., 2016; Haščík *et al*., 2017; Khan, 2017)**.

The use of propolis in the diets of Japanese quail improved feed intake, egg production, egg weight, and feed conversion rate; with this diet, the onset of egg laying started earlier **(Mehaisen *et al*., 2019)**. Quail chicks under heat stress showed better productive performance when propolis was added to their diets, as reported by **Mehaisen *et al.* (2017)**. Related to the egg quality, diets containing propolis may result in better shell weight, Haugh unit (HU), albumen height, yolk index **(Abdel-Kareem and El-Sheikh, 2017)**, egg weight, and shell thickness **(Mehaisen *et al*., 2019)** than diets without propolis (control group). The inclusion bee pollen at different levels affected the egg weight (quadratic), yolk index (linear), Haugh Unit and albumen index (cubically). On the other hand, supplemental bee pollen linearly decreased cholesterol, triglyceride and P values and linearly increased Mg value in the serum parameters **(Zekeriya and Hatice, 2020)**. **Arafa *et al.* (2016)** investigated the effect of dietary bee bread, bee pollen and their mixture as antioxidants on productive performance and functional properties of table eggs in Hy-line hens strain. Results indicated that all experimental diets produced greater egg number than control diets.

Awad *et al.* (2013) examined the effect of using BB as a natural supplement on productive and physiological performance of hens. They found that egg number and egg mass per hen and laying rate were significantly improved for the groups fed diets supplemented with different BB levels as compared to the control group during the overall experimental period (24–47 wks.), whereas the group fed with 0.5 g $BB.kg^{-1}$ had insignificantly higher values of these traits as compared to control group. All studied egg quality parameters were not significantly affected by used BB supplementation compared to the control.

The aim of study was to investigate the effect of supplementing the diets with different concentration of bee bread on the internal and external egg quality during laying period of Japanese quails.

MATERIAL AND METHODS

Animals and diet

The experiment was carried out on the Department of Small Farm Animals of the National Agricultural and Food Centre - Research Institute for Animal Production in Nitra. Japanese quails of meat line were included in the experiment. The quails were divided into four groups as follows: the control group received no additives (C), the experimental group E1 received bee bread at a dose of 2 g per 1 kg of feed mixture, experimental group E2 received 4 g of bee bread per 1 kg of feed mixture and E3 group 6 g bee bread per 1 kg of feed mixture. Bee bread was of Slovak origin (Medula Ltd., Bratislava). The groups were kept under the same conditions. The quails were reared using a cage technology, each cage was equipped with a feed disperser and water intake was ensured ad libitum through a self-feed pump up to whole experiment. **Table 1** shows the list of the ingredients and nutrient content of the basal diets formulated to provide the nutrient requirements of quails according to the recommended reference levels. The feed mixture was produced without any antibiotics and coccidiostats.

Egg quality

Japanese quails began to lay from the age of 7 weeks. The laying was recorded daily. We monitored the effect of the bee bread addition on the laying parameters of Japanese quail during the three months of laying (from 15 to 27 weeks of age). At the age of 16 (age 1), 20 (age 2) and 24 (age3) weeks of females, we performed an analysis of eggs. Total 360 eggs were analysed: 30 eggs from each group once a month for three consecutive months.

Egg weight was individually determined to 0.001 g accuracy using a laboratory scale Kern 572-32 (KERN, Balingen, Germany). Egg length (along the longitudinal axis) and egg width (along the equatorial axis) were measured with a micrometer. Egg shape index was calculated as the ratio of egg width to egg length (%) by method of **Anderson et al. (2004)**. After the eggs were broken, egg shells were washed with water and dried to clean the remaining albumen. Following Andreson´s procedure, shell weight (with membrane) was measured using a laboratory scale Kern 572-32 and the percentage proportion of the shell in the egg was calculated. Shell thickness (with membrane) was measured at the sharp poles, blunt poles and equatorial parts of each egg. Shell thickness was obtained from the average values of these three parts. The albumen weight was calculated as the difference between the egg weight, and the yolk and shell weight. The percentage proportion of the albumen in the egg was calculated. Albumen index (%) was determined according to **Alkan et al. (2010)** based on the ratio of the albumen height (mm) measurement taken with a micrometer to the average of width (mm) and length (mm) of this albumen with 0.01mm accuracy × 100. Individual Haugh Unit score (Haugh, 1937) was calculated using the egg weight and albumen height as follows **(Monira et al., 2003)**:

$$HU = 100 \log (H - 1.7w^{0.37} + 7.6)$$

H – observed height of albumen in mm

w – weight of egg in g

Yolk weight with 0.001 g accuracy was determined using a laboratory scale Kern 572-32 and its percentage proportion was calculated. Yolk index (%) calculated based on the ratio of the yolk height (mm) to the yolk width (mm) was measured according to Funk (1948) using micrometer with 0.01mm accuracy. Yolk colour was determined with La Roche scale (Hoffman–La Roche, Switzerland). Egg length (along the longitudinal axis) and egg width (along the equatorial axis) were measured with a micrometer. Egg shape index was calculated as the ratio of egg width to egg length (%) by method of **Anderson et al. (2004).**

Table 1 Composition of basal diet and nutrient content of feed mixture per kg of diet

Ingredients (%)	Feed mixture
Wheat	15
Maize	32
Soybean meal (48 % CP)	19.2
Fish meal (71% CP)	3
Malt flower	3
Rapeseed meal	7
Sunflower meal	4.5
Monocalcium phosphate	1
Fodder salt	0.3
Animal fat	4
Calcium carbonate	10
Premix of additives	1
Analysed composition ($g.kg^{-1}$)	
Crude protein	200
ME ($MJ.kg^{-1}$)	11.7
Fibre	60
Ash	160
Ca	35
P	5
Na	1.6
Lysine	11
Methionine + Cysteine	7.9
Linolic acid	10

Statistical analysis

Statistical analysis was done using the SAS 9.2 statistical software (2009). The GLM model was applied to study the influence of effects causing variation of individual egg quality parameter and age in Japanese quail. The following model was applied:

$Y_{ij} = \mu + D_i + A_j + e_{ij}$

where:
Y_{ij} – individual egg quality parameters
μ – intercept
D_i – fixed effect of diet (C, E1, E2, E3); $\Sigma i\ E = 0$
A_j – fixed effect of age (1, 2, 3); $\Sigma j\ A = 0$
e_{ij} – random error; $eij = N\ (0, I\sigma\ 2e)$
i=1 to 4
j=1 to 3

Differences among means were obtained using Tukey´s test for the same number of observations in each class of individual effects.

RESULTS AND DISCUSSION

The result of laying performance of control group (C) and group with addition of different concentration of bee bread in Japanese quails' diet are given in **Table 2**. As we can see from the table, we found the best laying indicators during three months laying period in the experimental group with the addition of 6 g per kg of feed. The control group had the lowest average laying performance both in the number of eggs per laying hen and in the laying intensity. Similar results were found by other authors with application of bee bread. **Arafa *et al.* (2016)** were investigated the effect of dietary bee bread (B), bee pollen (P) and their mixtures (BP) as antioxidants on productive performance and functional properties of table eggs in Hy-Line hens strain. They indicated that all the experimental diets produced greater egg number than control diet, but a clear improvement was observed in the hens fed with diet contained supplemented with 0.15% and 0.2% P and 0.1% B + 0.1%P. Egg mass per hen was significantly improved by the diet contained 0.2% P comparing with the control diet. The diets supplemented with 0.2% B, 0.15% P and 0.1% B recorded significantly lower values of triglycerides in fresh eggs yolk than control diet. The total cholesterol in stored yolks ranged from 18.02 to 45.99 $mg.dl^{-1}$ in the hens fed diets with BHT, 0.2% P, 0.1% B + 0.1% P, 0.15% B, 0.15% P and 0.2% B respectively while it was 63.79 $mg.dl^{-1}$ in control diet. The digestibility coefficient of ether extracts was significantly improved by the diet with 0.15% B and 0.15% P compared to the control diet. **Awad *et al.* (2013)** investigated the effect of supplementing bee bread (BB) as a growth promoter and antioxidant material at levels of 0, 0.5, 1.0 and 1.5 $g.kg^{-1}$ diet as well as a group supplemented with 250 mg ascorbic $acid.kg^{-1}$ as a positive control (PC) on laying performance and egg quality traits. Egg number and egg mass per hen and laying rate were significantly improved for the groups fed diets supplemented with different BB levels as compared to the control group during the overall experimental period (24–47 weeks), whereas the group fed 0.5 g $BB.kg^{-1}$ had insignificantly higher values of these traits compared to PC group.
Least squares means and standards errors for external and internal egg quality are given in **Table 3**. Egg weight is among the most important parameters not only for consumers, but for egg producers as well **(Genchev, 2012)**. In our experiment, average egg weight was significantly ($P \leq 0.05$) affected by diet and by age of quails. The quails of experimental group 2 had significantly lower egg weight than control and E3. At the beginning of the laying, the weight of the eggs (age 1) was significantly lower (12.84 g, versus 13.51 and 13.40 g). Similarly, the weight of the egg yolk and albumen were significantly lower at the beginning of laying. Gradually, the weight of the egg, yolk and albumen increased ($P<0.01$). The animal had significantly lower egg weight regardless of the experimental group at age of 16 week (age 1). We also found significant differences between the control group and experimental group 2 in some morphometric parameters of the egg (egg length and egg index). The eggs of experimental group 2 were shorter compared to the control group.

Table 2 Laying characteristics of Japanese quail

Month	Group C		Group E1		Group E2		Group E3	
	Average laying (piece/hen)	Laying intensity (%)	Average laying (piece/hen)	Laying intensity (%)	Average laying (piece/hen)	Laying intensity (%)	Average laying (piece/hen)	Laying intensity (%)
IX	23.92	79.72	26.18	87.27	22.25	74.17	25.27	84.24
X	23.75	76.61	25.73	82.99	27.71	89.40	28.40	91.91
XI	21.25	73.28	23.60	81.38	23.14	79.80	20.80	71.72
Together (90 days)	68.92	76.57	73.36	81.52	76.29	84.76	77.00	85.56

Legend: C – control group, E1– experimental group1 received bee bread at a dose of 2 g, E2 – experimental group 2 received bee bread at a dose of 4 g, E3 – experimental group3 received bee bread at a dose of 6 g per 1 kg of feed mixture

The most important quality traits of the egg shell are its thickness. We found in the thickness of the shell the most significant differences between the control group and the experimental groups. All experimental groups had significantly thinner shell in all measured parameters compared to the control one. In opposite to our work **Wang *et al.* (2007)** found that the laying hens supplemented with 1.5% bee pollen significantly increased shell thickness, yolk weight by about 9.22 and 6.89%, respectively than that of the control group. Similarly, **Mehaisen *et al.* (2019)** were found after the addition of propolis to the feed ration of Japanese quail significantly ($P \leq 0.001$) increasing the egg shell thickness. **Rizk *et al.* (2018)** and **Arpášová *et al.* (2012)** did not find significant influence of bee pollen on external egg quality of laying hens.
Japanese quail eggs have higher proportions of yolk than those from hens **(Fletcher *et al*., 1983; Zita *et al*., 2013)**. From yolk characteristics of eggs, we found significant differences only in yolk percentage and yolk colour between control group and E2 with addition of 4 g of bee bread. The control group had darker eggs (9.58ºHLR) than experimental groups (from 8.89 to 9.34ºHLR). The average yolk weight was insignificantly higher in experimental group than in control one. The highest relative yolk weight values may be due to the effects of antioxidants which may be attributed to release of vitellogenin from liver to improve those measurements **(Whitehead *et al*., 1998; Bowry and Ingold, 1999)**. These results are on the line with those obtained by **Ariana *et al.* (2011)** who reported that layer hens fed diet supplemented with natural antioxidant materials (α-tochopherol acetate, green tea powder and extract) produced eggs had a greater yolk weight and yolk index than those fed the control diet. The internal quality of albumen is assessed according to the content and consistency of the solid albumen bag by the albumen. The albumen index is an indicator of egg freshness **(Halaj and Golian, 2011)**. Haugh Unit expresses a more complex quality of protein in relation to its weight **(Halaj and Golian, 2011)**. As we can see from our experiment the addition of bee bread did not affect the quality of albumen.

From the indicators of the internal egg quality, we recorded significant differences between the control group and some experimental groups in the weight and width of the albumen. We also found a significant difference in the percentage of albumen and yolk between the control group and E2 ($P<0.05$). **Awad *et al.* (2013)** investigated the effect of supplementing BB on egg quality of chicken. All studied egg quality parameters were not significantly affected by feeding BB diets as compared to the control. In our experiment the albumen weight as well as the percentage of albumen was lower in all experimental groups compared to the control group. Similar results were observed by **Awad *et al.* (2013)**. Relative egg albumin weight was significantly decreased by feeding 1.0 (58.59±0.28% albumen weight) and 1.5 g $BB.kg^{-1}$ diets (58.35±0.49 %) than those fed PC diet. (59.97±0.54). They found significant differences between the groups in the width of the albumen. We did not find any significant differences in the Haugh Unit. Values ranged from 90.27 to 90.97. Similarly, **Awad *et al.* (2013)** did not find any significant differences in Haugh Unit in hens that received different concentrations of BB in their feed. HU values were lower (from 88.09 to 89.93).
In our work, we found a lower weight of the yolk and its percentage in the control group compared to the experimental groups. Similar results regarding the yolk content in hens feeding with bee bread were found in **Award *et al.* (2013)**.
Evaluating the impact of age on the quality of eggs, we recorded significant evidence in several indicators between the quality of eggs at the beginning of laying and the quality of eggs in the later laying period. From external indicators of egg quality, we recorded significant differences between individual age categories in egg weight and egg width **(Table 3)**. The weight and width of the egg have been shown to increase with age. **Genchev (2012)** also found similar results in the quails. During the experiment, the shape of eggs became more elongated (34.34 mm to 34.88 mm). Similar results confirmed **Genchev (2012)** in Japanese quails of Pharaoh and Manchurian golden lines. From the internal egg quality, we found significant differences in the weight of the albumin, where at the beginning of the laying the average weight was lower than at the end of the laying (from 7.60

g to 8.01 g). Similarly, the weight of the yolk increased with age (from 4.12 g to 4.40 g). Compared to **Genchev (2012)** we found the higher values of Haugh Unit (from 90.36 to 90.81 in our experiment), **Genchev (2012)** found HU from 88.99 to 89.04.

Tables 3 Least squares means and standards errors for egg quality

Effect	Bee bread					Age			
	Control	E1	E2	E3		1	2	3	
Trait		μ			s_{μ}		μ		s_{μ}
Egg weight (g)	13.41	13.16	12.96	13.46	0.121	12.84	13.51	13.40	0.105
			C:E2^{+}; E2:E3^{+}				1:2^{++}, 3^{++}		
Egg length (mm)	34.94	34.68	34.31	34.43	0.170	34.34	34.88	34.56	0.148
			C:E2^{+}				1:2^{+}		
Egg width (mm)	26.18	26.41	25.88	26.39	0.105	25.86	26.51	26.28	0.092
			E1:E2^{++}				1:2^{++}, 3^{++}		
Egg shape index	1.34	1.31	1.33	1.30	0.007	1.33	1.32	1.32	0.006
			C:E3^{+}						
Egg shape index (%)	75.10	76.27	75.55	76.71	0.419	75.43	76.16	76.13	0.363
			C:E3^{+}						
Shell weight (g)	1.15	1.14	1.14	1.10	0.012	1.12	1.15	1.12	0.010
			C:E3^{++}				1:2^{+}		
Shell percentage (%)	8.61	8.66	8.77	8.17	0.082	8.71	8.53	8.42	0.070
			C:E3^{++}; E2:E3^{++}				1:3^{+}		
Shell thickness - blunt ((µm)	219.03	213.69	212.47	210.93	0.941	213.61	214.05	214.43	0.815
			C:E1^{++}, E2^{++}, E3^{++}						
Shell thickness - sharp ((µm)	234.24	230.21	226.98	228.37	0.989	232.88	229.53	227.45	0.856
			C:E1^{++}, E2^{++}, E3^{++}				1:2^{+}, 3^{++}		
Shell thickness - equatorial ((µm)	225.54	221.48	219.01	219.24	0.901	222.27	221.08	220.62	0.781
			C:E1^{++}, E2^{++}, E3^{++}						
Shell thickness - average ((µm)	226.27	221.79	219.49	219.51	0.855	222.92	221.55	220.83	0.746
			C:E1^{++}, E2^{++}, E3^{++}						
Albumen weight (g)	8.02	7.74	7.60	7.98	0.092	7.60	8.01	7.89	0.080
			C:E2^{++}; E2:E3^{+}				1:2^{++}, 3^{+}		
Albumen percentage (%)	59.69	58.90	58.45	59.16	0.336	59.23	59.22	58.71	0.291
			C:E2^{+}						
Albumen height (mm)	4.86	4.82	4.92	4.91	0.056	4.88	4.89	4.86	0.048
Albumen width (mm)	43.16	42.26	43.02	43.16	0.255	43.75	41.52	43.43	0.221
			C:E1^{+}, E1: E3^{+}				1:2^{++}, 2:3^{++}		
Albumen index (%)	112.76	114.55	114.85	114.17	1.400	112.07	118.12	112.06	1.213
							1:2^{++}. 2:3^{++}		
Haugh Unit	90.27	90.29	90.97	90.61	0.305	90.81	90.44	90.36	0.264
Yolk weight (g)	4.24	4.28	4.25	4.38	0.046	4.12	4.38	4.40	0.040
							1:2, 3^{++}		
Yolk percentage (%)	31.70	32.56	32.78	32.66	0.272	32.15	32.24	32.88	0.236
			C:E2^{+}				1:3^{+}		
Yolk height (mm)	10.98	10.87	10.87	11.05	0.072	11.08	10.88	10.86	0.062
							1:2^{+}.3^{+}		
Yolk width (mm)	26.19	25.91	25.90	25.96	0.112	25.25	26.34	26.38	0.096
							1:2^{++},3^{++}		
Yolk index (%)	42.03	42.00	42.02	42.70	0.322	43.99	41.31	41.25	0.279
							1:2^{++}. 2:3^{++}		
Yolk colour (°HLR)	9.58	9.34	8.89	9.29	0.141	9.31	8.95	9.56	0.122
			C:E2^{++}				1:2^{+}. 2:3^{++}		

Legend: μ - least squares mean, s_{μ}- standard error, $^{+}$ - P<0.05, $^{++}$ - P<0.01

CONCLUSION

In our experiment, we evaluated the effect of the addition of bee bread in diet on Japanese quail's egg quality during laying. We recorded significant differences between the control group and some experimental groups in the weight and width of the albumen; in the percentage of albumen and yolk between the control group and E2. The control group had darker eggs than experimental groups. The most significant differences between the C and E groups were in eggshell thickness. All experimental groups had a thinner eggshell than C. We can conclude that the adding bee bread into Japanese quails' diets affected the laying parameters in favor of the experimental groups. The age of the quail had a more significant effect on the weight of both the whole egg and its individual components (albumen, yolk).

Acknowledgement: This publication was supported by the Operational Programme Integrated Infrastructure within the project: Sustainable smart farming systems taking into account the future challenges 313011W112, cofinanced by the European Regional Development Fund.

REFERNCES

Aliyzicioglu, Y., Deger, O., Ovali, E., Barlak,Y., Hosver, I., Tekelloglu, Y., Karaman, S.C. (2005). Effect of Turkish pollen and propolis extracts on respiratory burst for K-562 cell lines. *International Immunopharmacology*, 5(11), 1652-1657. https://doi.org/10.1016/j.intimp.2005.04.005

Alkan, S., Karabag, K., Galic, A., Karsli, T., Balcioglu, M. S. (2010). Effects of selection for body weight and egg production on egg quality traits in Japanese quails (*Coturnix coturnix japonica*) of different lines and relationships between these traits. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 16, 239-244. https://doi.org/10.9775/kvfd.2009.633

Al-Obaidi, F. A. and S. M. J. Al-Shadeedi (2017). Comparison some native fowls (chicken, mallard ducks quail and turkey) in components and chemical composition of the eggs in Iraq. *Al-Anbar Journal of Veterinary Science*, 10(1), 65–69.

Anderson, K. E., Tharrington, J. B., Cutris, P. A., Jones, F. T. (2004). Shell characteristics of eggs from historic strains of single comb white leghorn chickens and relationship of egg shape to shell strength. *International Journal of Poultry Science*, 3, 17-19.

Arafa, S. A., Omara, I. I., Beshara, M.M., Abdel-Azyme, M. G. (2016). Effect of adding bee bread as antioxidants on productive performance and funcyional properties of HY-Line hens strain. *Egypt. Poult. Sci.* 36 (IV), 905-929. ISSN: 1110-5623 (Print) – 2090-0570 (Online)
Ariana, M., Samie, A.l., Edriss, M. A., Jahanian, R. (2011). Effects of powder and extract form of green tea and marigold, and α-tocopheryl acetate on performance, egg quality and egg yolk cholesterol levels of laying hens in late phase of production. *J. of Med. Plants Res.*, 5, 2710-2716. https://doi.org/10.5897/JMPR.9000537
Arpašová, H., Kačaniová, M., Haščík, P., Sidlová, V. (2012). Effect of selected feed additives on internal quality parameters of table eggs. *Potravinarstro*, 6(4), 50-52. https://doi.org/10.5219/235
Awad, A. L., Beshara, M.M., Ibrahim, A.F., Fahim, H. N. (2013). Effect of using bee bread as a natural supplement on productive and physiological performance of local Sinai hens. *Egypt. Poult. Sci.*, 33(IV), 889-913. ISSN: 1110-5623 (Print) – 2090-0570 (On line)
Babaei, S., Rahimi, S., Torshizi, M. A. K., Tahmasebi, G., Miran, S. N. K. (2016). Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. *Veterinary Research Forum*, 7(1), 13-20.
Barajas, J., Cortes-Rodriguez, M., Rodríguez-Sandoval, E. (2012). Effect of temperature on the drying process of bee pollen from two zones of Colombia. *Journal of Food Process Engineering*, 35(1), 134–148. https://doi.org/10.1111/j.1745-4530.2010.00577.x
Barene, I., Daberte, I., Siksna, S. (2015). Investigation of bee bread and development of its dosage forms. *Medicinos Teorija Ir Praktika*, 21(1), 16–22. http://dx.doi.org/10.15591/mtp.2015.003.
Bobis, O., Marghitas, L. A., Dezmirean, D., Morar, O., Bonta, V., Chirila, F. (2010). Quality parameters and nutritional value of different commercial bee products. Bulletin of University of agricultural sciences and veterinary medicine Cluj–Napoca. *Animal science and biotechnologies*, 67, 1–2.
Bowry, V.W. and Ingold, K.U. (1999). The unexpected role of vitamin E (atocopherol) in the peroxidation of human low-density lipoprotein. *Acc. Chem. Res.*, 32, 27-34. https://doi.org/10.1021/ar950059o
Čeksterytė V., Balžekas J., Baltuškevičius A., Jurgevičius E., (2012). The use of beebread-honey mixture in the treatment of liver diseases in alcohol-dependent patients. *Chemical Technology*, 60(2), 62-66. https://doi.org/10.5755/j01.ct.60.2.1930
Fuenmayor, B., Zuluaga, C., Díaz, M., Quicazán de, C.M., Cosio, M., et al. (2014). Evaluation of the physicochemical and functional properties of Colombian bee pollen. *Revista MVZ Córdoba*, 19(1), 4003–4014.
Gardzielewska, J., Jakubowska, M., Tarasewicz, Z., Szczerbinska, D., Ligocki, M. (2005). Meat quality of broiler quail feed on feeds with different protein content. *Electronic Journal of Polish Agricultural Universities. Series Animal Husbandry*, 8(1), 13. ISSN 1505-0297, http://www.ejpau.media.pl/volume8/issue1/art-13.html
Genchev, A. (2012). Quality and composition of Japanese quail eggs (*Coturnix japonica*). *Trakia Journal of Sciences*. 10, 91-101. ISSN 1313-7050 (print), ISSN 1313-3551 (online)
Genchev A.G., Mihaylova, G., Ribarski, S. S., Pavlov, A., Kabanchiev, M. (2008). Meat Quality and Composition in Japanese Quails. *Trakia Journal of Sciences*, 6(4), 72-82.
Genchev A. G., Ribarski, S. S., Afanasjv, G. D., Bloghin, G. I. (2005). Fattening Capacities and Meat Quality of Japanese Quails of Faraon and White English Breeds. *Journal Central European of Agriculture*, 6(4), 495-500. https://doi.org/10.5513/jcea.v6i4.327
Halaj, M., Golian, J.: Vajce, biologické, technické a potravinárske využitie. Vydavateľstvo Garmond Nitra, 2011, 224 s. ISBN978-80-89148-70-7
Hanusová, E., Hanus, A., Hrnčár, C. (2018). Animal genetic resources of Slovak national poultry breeds. *Zborník abstraktov. Genetická konferencia GSGM*, 12.-14.9.2018 Bratislava, Univerzita Komenského v Bratislave. 2018, str. 57, ISBN 978-80-223-4545-3.
Hanusová, E., Hrnčár, C., Hanus, A., Oravcová, M. (2015). Effect of breed on some parameters of egg quality in laying hens. Acta fytotechn.zootechn., 18, 2015, http://www.fapz.uniag.sk/DOI: 10.15414/afz.2015.18.01.20-24
Haščík, P., Pavelková, A., Bobko, M., Trembecká, L., Elimam, I. O. E., Capcarová, M. (2017). The effect of bee pollen in chicken diet. *World's Poultry Science Journal*, 73(3), 643-650. https://doi.org/10.1017/S0043933917000435
Haugh, R. (1937). The Haugh unit for measuring egg quality. *U.S. egg & poultry magazine*, 43, 552-555, 573.
Hrnčár, C., Hanusová, E., Hanus, A., Bujko, J. (2014). Effect of genotype on egg quality characteristics of Japanese quail (*Coturnix japonica*). *Slovak Journal of Animal Science*, 47(1), 6-11, ISSN 1337-9984.
Ipek, A., Canbolat, O., Karabulut, A. (2007). The effect of vitamin E and vitamin C on the performance of Japanase Quails (Coturnix Coturnics Japonica) reared under heat stress during growth and egg production period. *Asian-Aust. J. Anim. Sci.* 20,252-256.
Khan, S. H. (2017). Recent advances in role of propolis as natural additive in poultry nutrition. *Journal of Apicultural Science*, 61(2), 167–183.
Kieliszek, M., Piwowarek, K., Kot, A. M., Błażejak, S., Chlebowska-Śmigiel, A., Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. *Trends in Food Science & Technology*, 71, 170-180. https://doi.org/10.1016/j.tifs.2017.10.021
Kocevski, D. et al. (2011). The influence of strain and age on some egg quality parameters of commercial laying hens.*Biotechnology in Animal Husbandry*, 27(4), 1649–1658.
Kročko, M., Čanigová, M., Bezeková, J., Lavová, M., Haščík, P., Ducková, V. (2012). Effect of nutrition with propolis and bee pollen supplements on bacteria colonization pattern in gastrointestinal tract of broiler chickens. *Animal Science and Biotechnologies*, 45(1), 63–67.
Maiorano, G., Elminowska-Wenda, G., Mika, A., Rutkowski, A., Bednarczyk, M. (2009). Effects of selection for yolk cholesterol on growth and meat quality in Japanese quail (*Coturnix coturnix japonica*). *Italian Journal of Animal Science*, 8(3), 457-466. https://doi.org/10.4081/ijas.2009.457
Markiewicz-Żukowska R., Naliwajko S.K., Bartosiuk E., Moskwa J., Isidorov V., Soroczyńska J., Borawska M.H. (2013). Chemical composition and antioxidant activity of beebread, and its influence on the glioblastoma cell line (U87MG). *Journal of Apicultural Science*, 57(2), 147-157.
Mehaisen, G. M. K., Ibrahim, R. M., Desoky, A. A., Safaa, H. M., El-Sayed, O. A., Abass, A. O. (2017). The importance of propolis in alleviating the negative physiological effects of heat stress in quail chicks. *PLoS ONE*, 12, e0186907. https://doi.org/10.1371/journal.pone.0186907
Mehaisen, G. M. K., Desoky, A. A., Sakr, O. G., Sallam, W., Abass, A. O. (2019). Propolis alleviates the negative effects of heat stress on egg production, egg quality, physiological and immunological aspects of laying Japanese quail. *PLoS ONE*, 14, e0214839. https://doi.org/10.1371/journal.pone.0214839
Monira, K. N., Salahuddin, M., Miah, G. (2003). Effect of breed and holding period on egg quality characteristics of chicken. *International Journal of Poultry Science*, 2, 261-263.
Mota-Rojas, D., González, V. A., Rojas, G. E., Aguilera, A. E., Flores-Peinado, S. C., Lemus-Flores, C., Olmos-Hernández, A., Becerril-Herrera, M., Cardona-Leija, A., Alonso-Spilsbury, M., Ramirez-Necoechea, R. (2007). Effect of Heat Stress during Transport and Rest before Slaughter, on the Metabolic Profile, Blood Gases and Meat Quality of Quail. *International Journal of Poultry Science*, 6(6), 397-402. https://doi.org/10.3923/ijps.2007.397.402
Nagai, T., Nagashima, T., Suzuki, N., & Inoue, R. (2005). Antioxidant activity and angiotensin I–converting enzyme inhibition by enzymatic hydrolysates from bee bread. *Zeitschrift für Naturforschung* C, 60(1–2), 133–138
Pavelková, A., Haščík, P., Capcarová, M., Kalafová, A., Hanusová, E., Tkáčová, J., Bobko, M., Čuboň, J., Čech, M., Kačániová, M. (2020a). Meat performance of Japanese Quails after the application of Bee bread powder. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 14, 2020, p. 735-743, ISSN 1337-0960(online), https://doi.org/10.5219/1330 s://dx.doi.org/10
Pavelková, A., Haščík, P., Kalafová, A., Capcarová, M., Čuboň, J., Bučko, O., Kačániová, M., Hanusová, E., Tkáčová, J., Bobko, M. (2020b). Chemical composition of muscle after bee bread application in the nutrition of Japanese Quails. *J Microbiol Biotech Food Sci.*, 2020b: 9 (4) 831-835. doi: 10.15414/jmbfs.2020.9.4.831-835, ISSN:1338-5178.
Peric. I., Zikie. D., Lukie. M. (2009). Application of alternative growth promoters in broiler production. *Biotechnology in Animal Husbandry*. 25(5-6), 387-397. http://www.doiserbia.nb.rs/img/doi/1450- 9156/2009/1450-91560906387P.pdf
Rizk, Y.S., Abuoghaba, A.A., Ismail, I. I. and Awadien, N.B. (2018). Egg production, egg quality traits and some hematological parameters of Sinai chicken strain treated with different levels of bee Pollen. Egypt. Poult. Sci., 38(II), 427-438. ISSN: 1110-5623 (Print) – 2090-0570 (Online)
Santos, T. C., Murakami, A. E., Fanhani, J. C., Oliveira, C. A. L. (2011). Production and reproduction of egg- and meat-type quails reared in different group sizes. *Revista Brasileira de Ciência Avícola*, 13(1), 9-14. https://doi.org/10.1590/s1516-635x2011000100002
SAS Institute Inc. 2009. SAS/STAT® 9.2 User's Guide, Second Edition, Cary, NC USA
Shale. B. A., Pasternak. H. (1993). Increment of egg weight with hen age in various commercial avian species. *British Poultry Science*. 34:915–924.
Tatli S. P. (2008) The Effects of dietary turkish propolis and vitamin C on performance, digestibility, egg production and egg quality in laying hens under different environmental temperatures. *Asian-Australas J Anim Sci.* 21(8), 1164-1170.
Tatli Seven, P., Seven, I., Yılmaz, M., Şimşek, U.G. (2008). The effects of Turkish propolis on growth and carcass characteristics in broilers under heat stress. *Animal Feed Science and Technology*, 146, 137-148.
Tatli Seven, P., Yılmaz, S., Seven, I., Çerçi, I. H., Azman, M.A., Yılmaz, M. (2009). The effect of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. *Acta Vet* Brno, 78, 75-83.
Tserveni-Gousi, A. S., Yannakopoulos, A. L. (1986). Carcass characteristics of Japanese quail at 42 days of age. *British Poultry Science*, 27(1), 123-127. https://doi.org/10.1080/00071668608416863
Vásquez, A., & Olofsson, T. C. (2009). The lactic acid bacteria involved in the production of bee pollen and bee bread. *Journal of Apicultural Research*, 48(3), 189–195.

Whitehead, C. C.; S. Bollengier-Lee; M. A. Mitchell and P. E. V. Williams (1998). Alleviation of depression in egg production in heat stressed laying hens by vitamin E. p. 576–578 in *Proceedings of 10th European Poultry Conference*, Jerusalem, Israel.

Zekeriya D. and Hatice K. (2020). Effect of Bee Pollen Supplemented Diet on Performance, Egg Quality Traits and some Serum Parameters of Laying Hens. *Pakistan J. Zool*., vol. 52 (2), 549-555. DOI: https://dx.doi.org/10.17582/journal.pjz/20181119101139

Zhang, K.Y., Yan, F., Keen, C. A., Waldroup, P. W. (2005). Evaluation of microencapsulated essential oils and organic acids in diets for broiler chickens. *International Journal of Poultry Science,* 4(9), 612-619. https://doi.org/10.3923/ijps.2005.612.619 81119101139

Zuluaga, C. M., Juan, C., Serrato, M., Quicazan, C. (2015).Chemical, nutritional and bioactive Characterization of Colombian bee-bread. *Chemical Engineering Transactions*, 43, 175-180, DOI: 10.3303/CET1543030