The effect of supplementing natural zeolite (NZ) on the laying characteristics and on the internal and external egg quality during the laying period of Japanese quails was studied. The laying and average eggs per female were lower in control (C) and the Z1 experimental groups (1% NZ) compare with the Z2 experimental group (2% NZ). The significant effect of the NZ addition in comparison with the C was found in the eggshell weight and yolk index. The eggshell weight of C (1.10±0.01 g) was significantly lower than Z1 (1.18±0.01 g). The quails of Z2 had significantly lower egg weight than Z1. The average egg weight was significantly affected by diet (P<0.05) and also by age of quails (P=0.001). At the beginning of the laying, the weight of the eggs (age 1) was significantly lower (12.07±0.12 g at the beginning, 12.38±0.12 g in the middle and 13.38±0.12 g in the end) (P<0.001). The albumen weight and Haugh unit were significantly influenced by the age of the females. The albumen weight increased with female age (from 6.88 to 8.10±0.09 g). The 1H was significantly lower (89.52±0.30) at the age 1 than in age 2 (90.50±0.30) and age 3 (90.57±0.30) (P<0.05). The yolk index was significantly affected by both diet and age of animals. The significant difference was between C and Z1 (P<0.05). The highest yolk index was found in youngest female (P<0.01). The egg quality was mainly affected by age of females. Diet affected investigated traits to a smaller extent. The effect of using natural zeolite (Clinoptilolite) in poultry diet on their performance are evidences in many works (Basha et al., 2016; Wawrzyniak et al., 2017; Morsy, 2018; Emam et al., 2019). Ozturk et al. (1998) by supplementing natural zeolite in various concentration (20, 40, 60, 80 g per 1 kg of diets) observed no significant dietary effects in terms of body weight, feed consumption, feed efficiency ratio, the number of eggs laid per hen, eggshell thickness, mortality or other criteria of egg quality (P>0.05). Dietary zeolite improves feed efficiency and egg production in laying hens (Samara, 2003). Moreover, Pavelic and Hadzija (2003) suggested that natural zeolite may have a beneficial effect against aflatoxicosis and other health disorders. Zeolite clinoptilolite is able to adsorb damaging toxins that can potentially reduce the performance of animals (Oğuz and Kurtoğlu, 2000), affect gut morphology, decrease pH, and lower pathogenic bacteria counts, which suggests that intestinal health improvement (Wu et al., 2013). Also, zeolite, being the adsorbents, eliminates a number of toxic substances (heavy metal salts, nitrates, nitrates, mycotoxins, radionuclides, metabolism products) from the organism. The zeolite in diets improves weight gains and feed conversion ratio (Debeic, 1994). The content of toxins, especially mycotoxins, in animal feed, especially poultry, is important from a health point of view. Thanks to its structure, natural zeolite is able to adsorb mycotoxins from feed and thus contributes to the improvement of animal health (Kermanshahi et al., 2011).
The important part of egg production as well as their quality is egg number. The egg quality is conditional upon a number of factors such as poultry species, genotype, nutrition, oviposition time, environment and age of laying hens. Johnston and Gous (2007) and Zita et al. (2009, 2012) showed that the egg weight increased with hen’s age. The egg weight was also affected by the age of the quails. Nazlíglí et al. (2001) and Orhan et al. (2001) found that egg weight increased with the age of the quail.

Quality of poultry products, especially eggs, is a very important issue for both the producers and consumers. Egg quality has been defined as the characteristics of an egg that affect its acceptability by the consumers. Egg quality is the more important price contributing factor in table and hatching eggs. Therefore, the economic success of a laying flock solely depends on the total number of quality eggs produced (Monira et al., 2003). Egg quality is composed of those characteristics of an egg that affects its acceptability to consumers, it is therefore important that attention is paid to the problems of preservation and marketing of eggs to maintain the quality (Hrnčár et al., 2014).

Gonzales (1995) were analysed the external and internal egg quality of Japanese quails during 6 months of lay. Egg weight, egg length, yolk height and yolk index increased as quail aged. Specific gravity, eggshell plus membrane thickness, egg shell index, albumen height and internal quality unit (IQU) decreased at the end of the experiment. Highest values of eggshell thickness (0.201 mm) and internal quality unit (69.68) obtained at 17 and 8 weeks of age, respectively, indicated that a better quality of egg was attained at the beginning of lay. The aim of the study was to investigate the effect of supplementing natural zeolite on the laying characteristics and 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. Japanese quails were kept in cage with three females per cage. A cycle of 14 h of light and 10 h of dark was used throughout the experiment. They were fed with commercial diets (TEKRO Nitra, Ltd. Slovak Republic). The quails were divided into three groups as follows: the control group received commercial diets without additives (C), the experimental group Z1 were fed a commercial diet enriched with supplement of 1 % natural zeolite and experimental group Z2 were fed a commercial diet enriched with supplement of 2 % natural zeolite. The animals had the feed and water at libitum. The natural zeolite (ZeoFeed) was supplied by Zeocom Company (Zeocom a.s., Bystré, Slovakia) from the quarry of Nižný Hrabovec, Slovakia. Zeolite used in the experiment had a particle composition was as follows: SiO\(_2\) – 70.98%, Al\(_2\)O\(_3\) – 11.72%, Fe\(_2\)O\(_3\) – 1.26%, CaO – 2.89%, MgO – 0.53%, K\(_2\)O – 3.25%, Na\(_2\)O – 0.56% and loss on ignition – 7.17%.

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 recommendations of the National Agricultural and Food Centre (2004). After the eggs were broken, eggshells were washed with water and dried in order to clean the remaining albumen. Shell thickness (with membrane) was measured at the sharp poles, blunt poles and equatorial parts of each egg. The albumen weight was calculated as the difference between the egg weight, and the yolk and eggshell weight. Individual Haugh unit score (Haugh, 1937) was calculated using the egg weight and albumen height as follows (Monira et al., 2003).

Yolk weight with 0.001 g accuracy was determined using a laboratory scale Kern 572-32 and its percentage proportion was calculated. Yolk colour was determined with La Roche scale (Hoffman et al., 2003). 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). Egg quality has been defined as the characteristics of an egg that affects its acceptability by the consumers; thus, egg quality is the more important price contributing factor in table and hatching eggs. Therefore, the economic success of a laying flock solely depends on the total number of quality eggs produced (Monira et al., 2003). Egg quality is composed of those characteristics of an egg that affects its acceptability to consumers, it is therefore important that attention is paid to the problems of preservation and marketing of eggs to maintain the quality (Hrnčár et al., 2014).

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**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
- \( \mu \) — intercept
- \( D_i \) — fixed effect of diet (C, Z1, Z2);
- \( \Sigma E = 0 \)
- \( A_j \) — fixed effect of age (1, 2, 3);
- \( \Sigma A = 0 \)
- \( e_{ij} \) — random error; \( e_{ij} \sim N(0, \sigma^2) \)

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 (average laying eggs per hen and laying intensity) of control group (C) and group with addition of different concentration of natural zeolite (Z1, Z2) in Japanese quails diet are given in table 2. As we can see from the table, the laying and average eggs per female was lower in control and Z1 experimental groups compared with the Z2 experimental group. These parameters were relatively balanced throughout the whole experiment. In agreement with works of Baumgartner and Hetényi (2001) and Hanušová et al. (2013), the laying of Japanese quails was the most intensive until 20 weeks of age. The laying gradually decreased in C and Z1 decreases with increasing age.

| Ingredients (%) | Feed mixture |
|-----------------|--------------|
| Wheat           | 15           |
| Maize           | 32           |
| Soybean meal    | 19.2         |
| Fish meal       | 3            |
| Malt flower     | 3            |
| Rapseased meal  | 7            |
| Sunflower meal  | 4.5          |
| Monocalcium phosphate | 1  |
| Fodder salt     | 0.3          |
| Animal fat      | 4            |
| Calcium carbonate | 10         |
| Premix of additives | 1   |

**Notes:** CP = crude protein; Ca = calcium; P = phosphorus; Na = natrium; ME\(_P\) = nitrogen-corrected metabolizable energy; MJ = megajoule; l'active substances per kilogram of premix: vitamin A 15 000 IU; vitamin E 20 mg; vitamin D 3 2 000 IU; riboflavin 6 mg; cobalamin 20 μg; Mn 60 mg; Zn 40 mg; Fe 40 mg; Cu 6 mg; I 11 mg; Se 0.2 mg.
Least squares means and standards errors for external and internal egg quality are given in table 3. The table shows that the eggs quality is more influenced by age than by the supplementing natural zeolite. The significant effect of the natural zeolite addition in comparison with the control group was found in the eggshell weight and the yolk index. The weight of eggshell of the control group (1.10±0.01 g) was significantly lower than the eggshell weight of experimental group Z1 (1.18±0.01 g) (P<0.05). We found just a few significantly differences in some egg quality traits between experimental groups Z1 and Z2. It follows that the natural zeolite concentration may have affected some indicators of egg quality (egg weight, eggshell weight, albumen weight).

### Table 2
Laying characteristics of Japanese quail

| Month | Average laying (piece/hen) | Laying intensity (%) |
|-------|-----------------------------|---------------------|
| VI    | 16.38                       | 81.92               |
| VII   | 24.58                       | 77.67               |
| VIII  | 20.31                       | 65.51               |
| IX    | 19.92                       | 66.39               |
| X     | 21.42                       | 69.09               |
| XI    | 14.58                       | 48.61               |
| XII   | 10.00                       | 32.26               |
| I     | 8.09                        | 26.10               |
| III   | 8.60                        | 29.66               |
| Total | 157.33                      | 53.33               |

(295 days)

The table shows that the eggs quality is more influenced by age than by the supplementing natural zeolite. The significant effect of the natural zeolite addition in comparison with the control group was found in the eggshell weight and the yolk index. The weight of eggshell of the control group (1.10±0.01 g) was significantly lower than the eggshell weight of experimental group Z1 (1.18±0.01 g) (P<0.05). We found just a few significantly differences in some egg quality traits between experimental groups Z1 and Z2. It follows that the natural zeolite concentration may have affected some indicators of egg quality (egg weight, eggshell weight, albumen weight).

### Table 3
Least squares means and standards errors for egg quality

| Trait                     | Control | Z1 | Z2 | 1   | 2   | 3   |
|---------------------------|---------|----|----|-----|-----|-----|
| Egg weight (g)            | 12.56   | 12.92 | 12.34       | 0.12 | 12.07 | 12.38 | 13.38 | 0.12 |
| Egg length (mm)           | 34.49   | 34.75 | 34.31       | 0.17 | 34.20 | 36.40 | 34.75 | 0.17 |
| Egg width (mm)            | 26.02   | 26.08 | 26.01       | 0.09 | 25.59 | 26.10 | 26.42 | 0.09 |
| Egg shape index           | 1.33    | 1.33  | 1.32        | 0.01 | 1.34  | 1.33  | 1.32  | 0.01 |
| Egg shape index (%)       | 75.54   | 75.09 | 76.02       | 0.36 | 74.94 | 75.54 | 76.17 | 0.36 |
| Eggshell weight (g)       | 1.10    | 1.17  | 1.08        | 0.01 | 1.08  | 1.11  | 1.17  | 0.01 |
| Eggshell percentage (%)   | 8.82    | 9.09  | 8.75        | 0.10 | 8.92  | 8.98  | 8.75  | 0.10 |
| Eggshell thickness - blunt (µm) | 214.71 | 213.75 | 212.22     | 0.95 | 218.11 | 212.63 | 209.94 | 0.95 |
| Eggshell thickness - sharp (µm) | 228.40 | 227.08 | 226.56     | 1.03 | 231.42 | 227.23 | 223.39 | 1.03 |
| Eggshell thickness - equatorial (µm) | 220.32 | 219.49 | 218.45    | 0.94 | 224.03 | 218.30 | 215.94 | 0.94 |
| Eggshell thickness - average (µm) | 221.14 | 220.11 | 219.08    | 0.93 | 224.52 | 219.39 | 216.42 | 0.93 |
| Albumen weight (g)        | 7.47    | 7.64  | 7.20        | 0.09 | 6.88  | 7.33  | 8.10  | 0.09 |
| Albumen percentage (%)    | 59.27   | 59.18 | 58.26       | 0.45 | 65.94 | 59.20 | 60.56 | 0.45 |
| Albumen height (mm)       | 4.78    | 4.69  | 4.74        | 0.05 | 4.55  | 4.77  | 4.89  | 0.05 |
| Albumen width (mm)        | 45.34   | 45.62 | 45.13       | 0.30 | 44.78 | 42.93 | 48.37 | 0.30 |
| Albumen index (%)         | 105.92  | 103.34 | 105.75    | 1.43 | 101.85 | 115.57 | 101.60 | 1.43 |
| Haugh Unit                | 90.46   | 89.72 | 90.41       | 0.30 | 89.52 | 90.50 | 90.57 | 0.30 |
| Yolk weight (g)           | 3.99    | 4.11  | 4.07        | 0.07 | 4.12  | 3.93  | 4.11  | 0.07 |
| Yolk percentage (%)       | 31.94   | 31.75 | 32.97       | 0.42 | 34.21 | 31.74 | 30.71 | 0.42 |
| Yolk height (mm)          | 10.60   | 10.51 | 10.31       | 0.09 | 10.42 | 10.30 | 10.70 | 0.09 |
| Yolk width (mm)           | 23.78   | 24.22 | 24.00       | 0.14 | 22.78 | 23.93 | 25.29 | 0.14 |
| Yolk index (%)            | 44.70   | 43.54 | 43.16       | 0.44 | 45.82 | 43.10 | 42.47 | 0.44 |
| Yolk colour (HHR)         | 6.03    | 6.18  | 6.26        | 0.11 | 5.67  | 4.88  | 7.92  | 0.11 |

**Legend:** µ - least squares mean, sµ - standard error, ‘-’ P<0.05, ‘**’ P<0.01, ‘***’ P<0.001
The 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 affected by diet (P<0.05) and also by age of quails (P<0.001). The Japanese quails of experimental group Z2 (adding of 2% natural zeolite) had significantly higher egg weight than experimental group Z1 (P<0.05). At the beginning of the laying, the weight of the eggs (age 1) was significantly lower (12.07±0.12 g at the beginning, 12.38±0.12 g in the middle and 13.38±0.12 g in the end of lay) (P<0.001). Similar results found Hanusová et al. (2021) by hens at 7, 8 and 13 months of age. The average egg weight was significantly increased with the age of the laying hens (P=0.0076). Nabarjan et al. (1991), Gonzales (1995), Altan, O., Oguz, I., Akbas, Y. (1998), Zita et al. (2012, 2013) found that the egg weight increased with the age of Japanese quails. Peric et al. (2017) found significant effect of hen age (P<0.01) on egg weight, shell strength, albumen height and Haugh unit (HU). Zita et al. (2012) show that egg weight was affected by the age of the hens and quails (overall means 61.13 and 12.52 g respectively). Despite frequent fluctuations, as the age of the laying hens and quails increased, the yolk index and yolk proportion increased as well, while the albumen index, eggshell strength and shell thickness decreased. The important indicator of the external quality of eggs is the eggshell. A lot of authors have reported that eggshell weight increases and eggshell thickness decreased with quails age (Gonzales, 1995; Altan, O., Oguz, I., Akbas, Y. 2001; Orhan et al., 2001; Zita et al., 2013). Similar results found Kermanshahi et al. (2011) in hens. The eggshell weight was significantly increasing with age, the lowest was at the beginning of the laying (1.08±0.01 g) and the highest at the end of the laying (1.17±0.01 g). Tumová et al. (2014) found in laying hens that the young hens had significantly lower eggshell weight (6.76±0.19 g) than the old one (7.74±0.19 g). They followed apart age also effect of strain, temperature and viscosity on the eggshell weight. They confirmed that only age had significant effect on these traits. The most important quality traits of the eggshell are its thickness. We did not find significant differences in eggshell thickness between groups. This is in agreement with works of Králík et al. (2006) and Emam et al. (2019). We found that eggshell thickness decreased with female age in all measurement sites. The average eggshell thickness significantly decreased from 224.52±0.93 µm at the beginning of the laying, to 219.39±0.93 µm in the middle of the laying up to the value of 216.42±0.93 µm. These agree with results of Hanusová et al. (2021). The thickness of eggshell was demonstrably thinner in all measured positions of the egg with increasing age of laying hens. The important internal egg quality traits are mainly the albumen weight, Haugh unit and yolk weight. The internal quality of albumen is assessed according to the content and consistency of the solid albumen bag by the albumen. The albumen index (AI) expresses the relationship of eggshell and albumen weight (Halaš, M., Golián, J., Vajce, biologická, technická a potravinárske využitie. Vydavateľstvo Garmon Nitra, 2011, 224 s.ISBN978-80-8948-10-7. Hanusová, E., Baumgartner, J., Heteňy, L., Hanus, A. (2013). Preparlja japonska. Vydalo Centrum výskumu živočíšnej výroby Nitra, v edicíci biblii CVŽ Nitra, ISBN 978-80-8948-1-100 np. Hanusová, E., Hanus, A., Hrnčár, C. (2021). Eggs quality of Oravka breed hens depending on hen’s age. Acta fytotechni zootechn, 24, 2021(Monothematic Issue: Problems and Risks in Animal Production): 11–14. https://doi.org/10.15414/afz.2021.24.mi.prp-11-14 Emam, K.R.S., Toraih, H.M., Hassan, A.M., El-Far, A.A.E., Morsey, A.S., Ahmed, N.A.L.H. (2019). 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Acta fytotechni zootechn, 24, 2021(Monothematic Issue: Problems and Risks in Animal Production): 11–14. https://doi.org/10.15414/afz.2021.24.mi.prp-11-14 Feeding natural substances to Japanese quails did not negatively influence laying performance of Japanese quails. The submitting of natural zeolite to the feed ration at a concentration of 1% or 2% did not negatively influence the internal and external egg quality of Japanese quails. The internal and external egg quality was more affected by age of laying female than by supplementing natural zeolite. Acknowledgments: The publication was supported by the project No. APVV-15-0477.

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