ABSTRACT
The objective of the present study was to determine the relationship between eggshell thickness and other eggshell characteristics in eggs produced in litter housing system and enriched cages. Eggs were collected from 200 birds of ISA Brown genotype at 40–42 weeks of age. Half of the birds were housed in enriched cages (750 cm²/hen, 10 hens/cage) and the other half were housed in littered pens (9 hens/m², 10 hens/pen). Eggs in each housing system were split into three categories varying in shell thickness: the first category (thin shells 0.28–0.30 mm), the second category (medium shells 0.33–0.36 mm) and the third category (thick shells 0.39–0.41 mm). Results indicated that eggshell parameters differ significantly according to eggshell thickness. Significant interaction of shell category and housing system was observed in eggshell strength. As expected, the eggshell strength was increased with eggshells becoming thicker. Moreover, eggs with the thickest shells from enriched cages had significantly stronger shells than those from litter system. Eggshell weight was significantly increased in the thick eggshell category being higher in enriched cages (7.23 g) than in litter system (5.14 g). The Pearson’s correlation coefficients showed a positive correlation between eggshell parameters and eggshell thickness in both housing systems. Moreover, the correlation between eggshell thickness and eggshell strength was higher on litter (0.64, \(p < 0.001\)) in comparison with enriched cages (0.48, \(p < 0.001\)). Results of the present study indicated that in thin shells, housing system plays an important role in determining the eggshell strength.

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Introduction
The eggshell of laying hens still remaining one of the greatest interest of researchers, regarding to the economic losses of cracked and damaged eggs which accounts for 6 to 8% of total egg production (Hamilton et al. 1979); it also provides a pass way for micro-organisms penetration into the eggs. Therefore, improving overall eggshell quality would have a significant economic impact on egg production industry. The function of the eggshell is maintained by its structure as a complex of bio-ceramic material with 95% calcium carbonate (Nys et al. 1991).

Eggshell quality traits play an important role because only eggs with an intact shell are considered for hatching or as table eggs. Therefore, if the eggshell quality is guaranteed, the egg industry could increase the number of eggs produced by each hen housed. Eggshell thickness play a major role of these parameters of the eggshell. However, there is no direct effect of this parameters on the other eggshell properties.

Eggshell quality parameters might differ between housing systems (Tůmová and Ebeid 2005). Lichovniková and Zeman (2008) reported that heavier eggshells were produced in conventional cages compared to floor system and enriched cages. In contrast, the heaviest eggshells in litter system in comparison with conventional and enriched cages were observed by (Tůmová et al. 2011). Pavlovski et al. (2001) compared the effect of litter, free-range and cages housing systems on eggshell thickness and obtained thicker shells on litter eggs compared to free-range. Leyendecker et al. (2001) found that, lower eggshell thickness was obtained in eggs produced in cages while free-range eggs had the highest values of traits. Moreover, several studies reported that shell thickness was lower in eggs from cages than in litter housing.
system (Hidalgo et al. 2008; Tůmová et al. 2011; Ledvinka et al. 2012). Similarly, ambiguous results in different housing systems were observed for eggshell strength. Tůmová et al. (2011) observed the highest eggshell strength in cages system (4744 g/cm²) compared to litter system (4651 g/cm²). Similarly, Ledvinka et al. (2012) and Englmaierová et al. (2014) found stronger shells in cages compared to litter system. These results indicated that eggshell thickness and strength do not have the same trends in each housing system. Therefore, it is important to compare the relationship between eggshell quality characteristics according to housing system.

The differences in the physical variables like eggshell weight and thickness could explain nearly 60% of the eggshell strength variation (Frank et al. 1965). Several recent articles paid more attention to detect the relationship between shell thickness and strength. Yan et al. (2014) studied the effect of uniformity of eggshell thickness (a new parameter to evaluate eggshell quality, it is defined as the reciprocal of coefficient of variation of eggshell thickness from multiple positions) on eggshell quality and reported a positive correlation between shell thickness and breaking strength ($r = 0.319$) and static stiffness ($r = 0.425$), while they detected negative correlation with fracture toughness ($r = 0.472$). They also indicated that, eggs with thin but more uniform eggshell were stronger than those with thick but less uniform eggshell. Moreover, Kibala et al. (2015) reported a higher genetic correlations (0.8) between eggshell strength and its thickness making shell thickness as a selection index candidate element. However, thicker eggshells do not guarantee stiffer or stronger eggs (Bain 2005). Tůmová et al. (2011) and Ledvinka et al. (2012) indicated that, although shell thickness was lower in eggs produced in cages, eggshell strength was higher and this effect might be related to the ultra-structural features of the shells in cages eggs which presumably support the eggshell strength. Nevertheless, it might be assumed that housing system affect eggshell microstructure resulting different eggshell thickness and strength. Also, housing system had a significant effect on pores density (Ketta and Tůmová 2016).

The previous studies were conducted on one housing system and there is a question, whether housing systems affect eggshell characteristics when eggs differ in thickness? Therefore, the objective of the present study was to determine the relationship between eggshell thickness and other eggshell measurements in eggs produced in litter housing system and enriched cages.

Materials and methods

**Experimental design and diets**

Eggshell quality parameters were evaluated in an experiment with 200 laying hens of ISA Brown at the age of 40–42 weeks. Laying hens were housed in enriched cages (100 hens, 750 cm²/hen, 10 hens/cage) and in littered pens with wood shavings (100 hens, 9 hens/m², 10 hens/pen).

According to eggshell thickness, eggs were split into three categories: the first category (thin shells; 0.28–0.30 mm, 377 eggs from enriched cages and 312 eggs from litter system), the second category (medium shells; 0.33–0.36 mm, 497 eggs from enriched cages and 291 eggs from litter system) and the third category (thick shells; 0.39–0.41 mm, 405 eggs from enriched cages and 424 eggs from litter system).

Laying hens in both housing systems were fed identical commercial feed mixture with 15.37% crude protein, 11.58 MJ of metabolisable energy, 3.48% calcium and 0.56% of total phosphorous. Feed and water were supplied *ad libitum*. The daily photoperiod consisted of 14 h light, with an intensity of 10 lx at bird head level. The environmental conditions were kept according to the method described by Skrivan et al. (2015).

**Eggshell quality assessments**

Eggs were analysed every week three days in a row, and individually weighed, length and width of each egg were measured for egg shape index calculation ($\text{width/length} \times 100$). Eggshell strength was determined by the shell-breaking method using a QC-SPA device (TSS, England). After the eggs were broken, eggshell thickness was measured with a QCT shell thickness micrometer (TSS, England) at the equatorial area after removal of shell membranes. Eggshell weight was determined after drying according to (Englmaierová et al. 2015) and the eggshell percentage was calculated. The surface area of each egg was determined using the equation reported by Thompson et al. (1985): \( \text{Egg surface area} = 4.67 \times (\text{egg weight})^{2/3} \). Eggshell index was calculated according to the followed equation: Eggshell index = (shell weight/shell surface) × 100 (Ahmed et al. 2005).

**Statistical analysis**

Data were statistically analysed using two-way analysis of variance (housing × shell thickness) using GLM procedure of SAS (SAS 2003). The relationship between eggshell parameters was evaluated by estimating Pearson’s correlation coefficient.
Results

Interactions between shell thickness and housing system

Significant interaction of eggshell thickness category and housing system was detected for eggshell strength \((p < .05)\), eggshell weight \((p < .05)\), shell percentage \((p < .001)\) and eggshell index \((p < .001)\) and eggshell index \((p < .001)\) and eggshell index \((p < .001)\) and eggshell index \((p < .001)\) and eggshell index \((p < .001)\). Egg weight was significantly affected by shell thickness category \((p < .001)\), eggs become heavier with increasing shell thickness. In enriched cages, eggs were significantly \((p < .05)\) heavier than in litter system. Eggshell strength were affected by the interaction between eggshell thickness category and housing system \((p < .034)\). Eggshell strength did not differ according to housing system in the thick and medium category, however, in the thin category, eggs from enriched cages had significantly stronger shells in comparison with litter system. Also eggshell thickness category affected shell strength \((p < .001)\), whereas shells being stronger in the thick category. Also, eggshell weight was significantly affected by the interaction of the shell thickness category and housing system. The highest eggshells weight were observed in thick shells category in enriched cages and the lightest eggshells were detected in thin shells category in litter system. Moreover, data showed that eggshell weight significantly differed in enriched cages and in litter system in the thick and the thin shells categories, whereas in the medium shell category was not affected. Eggshell weight was increased with eggshell thickness category \((p < .001)\). Similar trends of the significant interaction of the eggshell thickness category and housing system were observed in eggshell percentage \((p < .001)\) and eggshell index \((p < .001)\). In contrast with the shell weight, eggshell percentage and eggshell index were not significantly \((p < .05)\) affected by housing system. However, the interaction showed a higher values of traits in cage system compared to litter mainly in the thin category. Eggshell surface significantly increased with the shell thickness category.

Pearson’s correlation coefficients of eggshell parameters

The Pearson’s correlation coefficients of eggshell parameters of eggs produced in enriched cages (Table 2) and in litter system (Table 3) indicated positive correlations among eggshell thickness and the other eggshell parameters. Also, in other eggshell measurements, higher correlations were observed in litter system than in enriched cages except the
eggshell surface area. In enriched cages, negative correlation ($p < .05$) between eggshell strength and eggshell surface area was detected, while, in litter system positive correlation ($p < .01$) was obtained. A negative correlation ($p < .001$) was detected between eggshell percentage and eggshell surface area with higher values in litter system than in cages.

**Discussion**

The obtained results revealed that the different shells thickness categories significantly affected the other eggshell quality characteristics. The egg weight significantly increased when the eggs become thicker. The increasing in egg weight might be related to higher eggshell weight which also increased with the eggshell thickness category. This relationship was estimated by correlations between egg weight and eggshell weight (0.64 in enriched cages and 0.56 in litter system). Lower correlations in litter system might be assumed to be affected by significantly lower egg weight in litter system. Results of the present study indicated a significant interaction of shell thickness category and housing system for eggshell strength. The eggshell strength significantly increased as the eggshells become thicker; with different values between eggs produced in enriched cages and in litter system especially in the thin shell category, while shell strength did not differ between litter and enriched cages in the medium and thick shell categories. These results are in agreement with Kibala et al. (2015) who reported a positive genetic correlations between eggshell strength and its thickness. On the other hand, Tatara et al. (2016) revealed a negative correlation between eggshell thickness and eggshell strength, indicating that mechanical endurance of the eggshell is not simply affected by its thickness but other factors such as mineral density, mineral content and spatial micro architectural arrangement contribute to this characteristic. Results of the present study indicated a significantly positive correlation between egg shape index and eggshell strength in both housing systems. The larger, and rounder eggshells have the higher resistance to breaking forces. These results are in agreement with the findings of Anderson et al. (2004) and Blanco et al. (2014) who reported positive correlation between eggshell strength and egg shape index index. Therefore, it is necessary to monitor egg shape to maintain an optimal form for stronger eggshells.

The interaction of shell thickness category and housing system was also observed for eggshell percentage. The thin and thick shells categories showed big differences between enriched cages and litter system for eggshell percentage, while the medium shell category did not differ. These results might be related to the uniformity of eggshell thickness as Yan et al. (2014) reported that eggs with thin but more uniform eggshell showed better shell measurements than those with thick but less uniform eggshells. A higher negative correlation between eggshell percentage and

### Table 2. Pearson’s correlation coefficients between eggshell parameters of eggs produced in cages ($n < 1279$).

|                      | Egg weight | Eggshell strength | Eggshell thickness | Eggshell weight | Egg shape index | Eggshell percentage | Eggshell surface area |
|----------------------|------------|-------------------|--------------------|-----------------|-----------------|---------------------|-----------------------|
| Eggshell strength    | $-0.12^*$  | 0.15**            | 0.64***            | 0.56            | 0.12*           | 0.45***             | 0.99***               |
| Eggshell thickness   | 0.20***    | 0.64***           | 0.56***            | 0.56*           | 0.06            | 0.53***             | 0.99***               |
| Eggshell weight      | 0.56***    | 0.62***           | 0.85***            | 0.56*           | 0.16*           | 0.21***             | 0.94***               |
| Egg shape index      | 0.06       | 0.18**            | 0.05               | 0.05            | 0.06            | 0.82***             | 0.09                  |
| Eggshell percentage  | $-0.21^*$  | 0.53***           | 0.74***            | 0.65***         | 0.21***         | 0.83***             | $-0.02$               |
| Eggshell surface area| 0.99***    | 0.16**            | 0.21***            | 0.56***         | 0.06            | 0.82***             | 0.95***               |
| Eggshell index       | 0.09*      | 0.60***           | 0.82***            | 0.83***         | 0.09*           | 0.82***             | 0.09                  |

*p < .05.
**p < .01.
***p < .001.

### Table 3. Pearson’s correlation coefficients between eggshell parameters of eggs produced on Litter ($n < 1027$).

|                      | Egg weight | Eggshell strength | Eggshell thickness | Eggshell weight | Egg shape index | Eggshell percentage | Eggshell surface area |
|----------------------|------------|-------------------|--------------------|-----------------|-----------------|---------------------|-----------------------|
| Eggshell strength    | 0.16**     | 0.20***           | 0.64***            | 0.56            | 0.06            | 0.53***             | 0.99***               |
| Eggshell thickness   | 0.56***    | 0.62***           | 0.85***            | 0.56*           | 0.16*           | 0.21***             | 0.94***               |
| Eggshell weight      | 0.06       | 0.18**            | 0.05               | 0.05            | 0.06            | 0.82***             | 0.09                  |
| Egg shape index      | $-0.21^*$  | 0.53***           | 0.74***            | 0.65***         | 0.21***         | 0.83***             | $-0.02$               |
| Eggshell percentage  | 0.99***    | 0.16**            | 0.21***            | 0.56***         | 0.06            | 0.82***             | 0.95***               |
| Eggshell surface area| 0.09*      | 0.60***           | 0.82***            | 0.83***         | 0.09*           | 0.82***             | 0.09                  |

*p < .05.
**p < .01.
***p < .001.
egg weight was found in litter system compared to enriched cages. While, the correlation between eggshell thickness; eggshell weight and eggshell percentage were highly positive in litter system. The eggshell index was similar to eggshell percentage being significantly different between enriched cages and litter systems in the thin and thick shells category only with higher values in enriched cages than in litter system. Also the correlation between eggshell thickness; eggshell weight and eggshell index were positively higher in litter system than in enriched cages. The different results between both housing systems might be assumed as the greater chance of eggshell contamination exists in litter system compared to enriched cages. Eggshell index is related to shells crystal size and lower values indicate larger crystals which causes lower eggshell strength (Ahmed et al. 2005). Based on the lower values of the eggshell index in the thin category, it might be assumed that the thin shells mainly in litter system are created from larger crystals which result to lower eggshell strength. It seems that eggshell structure in the thin shells plays more important role than in eggs with thick shells mainly in litter housing system.

Conclusions
In conclusion, the current study results investigated the important relationship between eggshell thickness and other eggshell parameters. As expected, the eggshell strength increased with eggshells becoming thicker. Moreover, eggs with the thickest shells from enriched cages had significantly stronger shells than those from litter system. These results indicated that in the thin shell thickness, housing system plays an important role in the relationship to strength which might be related to the crystals size and orientation as the major determinant of shell thickness and strength. More attention should be paid to the egg shape to ensure better shell quality characteristics.

Disclosure statement
No potential conflict of interest was reported by the authors.

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