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Eggshell Characteristics and Cuticle Deposition in Three Laying Hen Genotypes Housed in Enriched Cages and on Litter

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ABSTRACT

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The objective of the present study was to compare the eggshell characteristics and cuticle deposition of Lohmann Brown, Hy-Line Silver Brown, and Isa Brown layers kept in two different housing systems. The three laying hen genotypes were housed in enriched cages (100 hens, 750 cm^2/hen, 10 hens/cage) and in littered pens (100 hens, 9 hens/m^2, 10 hens/pen). The experiment was carried out in weeks 40–56 of hens age. Non-significant interactions of genotype and housing system for eggshell quality parameters and cuticle deposition were detected in this study. Egg weight was significantly affected by genotype ($P \leq 0.001$) and housing system ($P \leq 0.043$). The heaviest eggs were laid by Lohmann Brown, while the lightest eggs were produced by Hy-Line Silver Brown. Eggshell strength was not affected by genotype and housing system, however, genotype had a significant effect on eggshell thickness ($P \leq 0.033$). Isa Brown eggs had thicker eggshells compared to Lohmann Brown and Hy-Line Silver Brown. However, a non-significant effect of housing system on eggshell thickness was observed. Eggshell percentage was significantly affected by both genotype and housing system. Genotype of laying hens had a significant effect on cuticle deposition; significantly higher cuticle deposition was observed in Lohmann Brown eggs ($P \leq 0.001$). It could be concluded that genotype had a significant effect on eggshell quality parameters and cuticle deposition. However, the housing system effect was less important in these characteristics.

Keywords: eggshell quality; cuticle quality; genotype; housing system

Eggshell quality is considered a major concern in the egg industry because of the economic losses related to the incidence of eggshell defects. In egg industry, the eggshell is essential to provide the shape of the egg and as a container of the internal egg components protecting it from environmental conditions. However, these features of the eggshell are reserved by its unique structure. Mineralized eggshell is formed mainly of calcium carbonate (96%); the remaining components include organic matrix (2%), magnesium, phosphorus, and a variety of trace elements (Nys et al. 2004). From the inside outwards, the eggshell comprises of shell membranes and true shell that includes mammillary layer, palisade layer, vertical layer, and cuticle (Gautron et al. 2014). Eggshell cuticle is a very thin organic layer covering the eggshell surface and plugs the shell pores openings to limit water, gases, and bacterial penetration through the eggshell (De Reu et al. 2006). It is composed of inner

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calcified and outer non-calcified water insoluble layers which are deposited directly onto the vertical crystal layer of the eggshell (Kusuda et al. 2011). The structure of the eggshell is often expressed by eggshell quality characteristics including eggshell strength and thickness. These characteristics are known to be affected by several internal and external factors such as genotype of laying hens and housing systems which are considered the most important (Ketta and Tumova 2016).

Commercially available genotypes differ mainly in egg weight, shell thickness, and strength. Thus, selecting the hen genotype which provides better eggshell quality characteristics is a very important issue to be considered. The differences in egg weight according to variable hen genotypes was investigated by Zita et al. (2009) who reported a significantly higher egg weight in eggs from Hisex Brown compared to Isa Brown and Moravia BSL. Moreover, egg weight differences between Lohmann LSL and a traditional breed the Czech Hen were obtained by Tumova et al. (2016).

The eggshell strength is of utmost importance for egg producers, as the lower strength causes higher percentage of broken eggs increasing the economic losses. Zita et al. (2009) reported significantly stronger shells from Isa Brown eggs compared to Hisex Brown and the tinted-egg hybrid Moravia BSL. On the other hand, non-significant differences in shell strength were determined by Tumova et al. (2007) in variable dominant strains. The effect of hen genotype on eggshell thickness was confirmed in several studies (Singh et al. 2009; Tumova et al. 2011). Eggshell percentage might be affected by hen genotype as it differs in egg weight and eggshell weight. Tumova et al. (2016) reported that higher shell percentage was observed in Lohmann LSL eggs compared to Czech Hen. However, Basmacioglu and Ergul (2005) described a non-significant effect of genotype on shell percentage. The deposition of cuticle is influenced by a number of factors including age, genotype, egg washing, and stress. Samiullah and Roberts (2014) suggested that brown eggs have the ability to prevent bacterial penetration more than white eggs which might be related to higher cuticle deposition in brown eggs. However, studies on the effect of genotype on cuticle deposition are limited and need more investigations.

The housing system is considered as a very important factor affecting eggshell quality. Unsuitable housing systems might increase the number of broken eggs, diseases, and general stress which consequently affect the shell parameters, mainly strength.

There is a large degree of variability in the research findings on the effects of housing system on egg weight and eggshell quality parameters providing unclear indication of which production system maintains eggs with the best shell quality (Holt et al. 2011). The effect of housing system on egg weight was studied by Lichovnikova and Zeman (2008) who observed higher egg weights were produced from hens housed in cages, whereas Tumova and Ebeid (2005) reported heavier eggs were produced from litter system.

Studying the effect of housing system on eggshell strength, Tumova et al. (2011) obtained stronger eggshells in the cage housing system compared to litter. Similarly, Ledvinka et al. (2012) and Englmaierova et al. (2014) found stronger shells in cages than in litter system. Studies on the effect of housing system on cuticle deposition are very limited and need more investigations. It is hypothesized that the genotype of laying hens and the housing system might affect eggshell quality characteristics and cuticle deposition. Therefore, the aim of the present study was to evaluate the differences in eggshell quality characteristics and cuticle deposition of laying hen genotypes housed in cages and in litter system.

MATERIAL AND METHODS

Animals and conditions. Lohmann Brown, Hy-Line Silver Brown, and Isa Brown laying hens at the age of 40–56 weeks were housed in enriched cages (100 hens, 750 cm^2/hen, 10 hens/cage) and in littered pens (100 hens, 9 hens/m^2, 10 hens/pen). The environmental conditions were similar to those described by Skrivan et al. (2015). Laying hens in both housing systems were fed an identical commercial feed mixture with 15.37% crude protein, 11.58 MJ of metabolizable energy, 3.48% of 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. During the experiment, eggs were collected in four-week intervals to be 660 eggs in total (20 eggs/genotype/housing system) and divided into two groups; 330 eggs were used for

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analyzing the eggshell quality characteristics and the other 330 eggs were used to estimate cuticle deposition.

Eggshell quality assessments. Freshly laid 330 eggs were individually weighed, length and width of each egg were measured for the egg shape index calculation (width/length × 100).

Eggshell strength was determined by the shell-breaking method using a QC-SPA analyzer (Technical Services and Supplies Ltd., UK). Eggshell thickness was measured with a QCT shell thickness micrometer (Technical Services and Supplies Ltd.) at the equatorial area after removal of shell membranes. Eggshell weight was determined after drying according to Englmaierova 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):

$$\text{Egg surface area} = 4.67 \times (\text{egg weight})^{2/3}$$

Estimation of cuticle deposition. Totally 330 eggs were used for cuticle estimation by the method of Roberts et al. (2013). Eggshells were individually soaked in a MST cuticle blue stain (MST Technologies Ltd., UK) for 1 min and rinsed in tap water 3 times to remove the excess stain. The eggshell colour was measured using a hand-held spectrophotometer CM-2600d (Konica Minolta Inc., Japan) which works on the L*a*b* colour space system. L* has a maximum of 100 (white) and a minimum of 0 (black). For a*, green is towards the negative end of the scale and red towards the positive end. For b*, blue is towards the negative end and yellow towards the positive end of the scale (Roberts et al. 2013). The reading was taken 3 times per location at 3 locations around the equator of each egg and an average was recorded.

The recorded average of L*, a*, and b* values, before and after staining, was used to calculate ΔE^*_{ab}:

$$\Delta E^*_{ab} = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

A higher ΔE^*_{ab} denotes a higher staining affinity and hence more cuticle coverage (Leleu et al. 2011).

Statistical analysis. The experiment data were evaluated with ANOVA, two-way analysis of variance using the GLM procedure of the SAS software (Statistical Analysis System, Version 9.4., 2013). The model included the effects of genotype and housing system. A value of $P < 0.05$ was considered significant for all measurements.

RESULTS AND DISCUSSION

Results of the present study showed the differences in egg weight according to the genotype of laying hens and the housing system. As shown in Table 1, Lohman Brown produced heavier ($P \leq 0.001$) eggs compared to Isa Brown and Hy-Line Silver Brown. The effect of genotype on egg weight was reported by Tumova et al. (2011) and Ledvinka et al. (2012) who detected variable egg weights from different hen genotypes. The egg weight was significantly affected by housing systems. Eggs were heavier in enriched cages ($P \leq 0.043$) compared to the litter system (Table 1). These results correspond with the findings of Englmaierova et al. (2014) who detected heavier eggs in the cage system compared to litter. On the other hand, Tumova and Ebeid (2005) and Pistekova et al. (2006) detected heavier eggs in litter systems compared to conventional cages. These conflicting results might be related to different experimental conditions and management. The eggs of Isa Brown hens were longer than those from Lohmann Brown and Hy-Line Silver Brown resulting in significantly higher egg shape index values ($P \leq 0.019$).

In literature, eggshell quality characteristics were more affected by genotype than by housing system. In the present study, eggshell strength was not significantly affected by either hen genotype or housing system (Table 1). However, differences in eggshell strength due to laying hen genotype were reported in previous studies (Zita et al. 2009; Ledvinka et al. 2012). Regarding the housing systems, no significant differences in shell strength were observed between eggs produced in litter system and cages (Pistekova et al. 2006). On the other hand, Englmaierova et al. (2014) revealed stronger eggshells produced in cages compared to litter housing system. Thus, it can be assumed that this contrast in results of eggshell strength might be related to the structure of the eggshell, especially the size and orientation of shell crystals or the mineral content of the eggshells. The relationship between eggshell strength and eggshell thickness is very important to overall shell measurements and might differ according to the thickness of the shell. Kibala et al. (2015) observed a genetic correlation between eggshell strength and its thickness was around 0.8, making the shell thickness a selection index candidate element. Ketta and Tumova (2017) indicated that

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Table 1. Effect of genotype and housing system on eggshell quality measurements and cuticle deposition

Genotype	Housing system	Egg weight (g)	Egg shape index (%)	Shell strength (g/cm^2)	Shell thickness (mm)	Shell percentage (%)	Shell surface (cm^2)	ΔE^*_{ab}
Lohmann Brown	cage	69.24	74.71	4027	0.358	9.48	76.67	51.24
	litter	66.60	75.85	4165	0.367	9.86	76.68	50.04
Hy-Line Silver-Brown	cage	62.55	76.31	4102	0.361	9.84	73.57	42.50
	litter	61.42	75.70	3811	0.359	10.08	72.66	40.51
Isa Brown	cage	67.41	76.61	4081	0.369	9.86	77.30	45.29
	litter	67.61	76.44	3925	0.375	10.09	77.46	42.05
RMSE		4.53	2.77	831	0.031	0.79	3.47	10.19
Genotype		0.001	0.019	0.561	0.033	0.026	0.001	0.001
Housing		0.043	0.736	0.339	0.287	0.006	0.042	0.105
Genotype * housing		0.192	0.124	0.274	0.479	0.823	0.202	0.807

ΔE^*_{ab} = calculated single score of colour difference for estimation of the degree of staining (Leleu et al. 2011), RMSE = root mean square error

the eggshell strength was significantly increased as the eggshells became thicker. Also there were 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 (Ketta and Tumova 2017). In the present study, Isa Brown produced the thickest ($P \leq 0.033$) eggshells in comparison with Lohmann Brown and Hy-Line Silver Brown. Hence, the eggshell thickness was significantly affected by laying hen genotypes in spite of the non-significant effect on eggshell strength. This finding might be explained by Tatara et al. (2016) who indicated that the mechanical endurance of the eggshell is not simply affected by its thickness but by other factors, e.g. mineral density, mineral content, and spatial micro architectural arrangement contribute to this characteristic.

No significant effect of housing system on eggshell thickness was detected in the present study (Table 1). These results are in agreement with Van Den Brand et al. (2004) who found no differences in eggshell thickness in eggs from cages and outdoor system. On the other hand, Tumova et al. (2016) reported a higher eggshell thickness in cages than in litter systems between Lohmann LSL and the Czech Hen. These differences between studies might be explained by different laying hen age or the interaction of genotype and housing system. As shown in Table 1, eggshell percentage was significantly affected by hen genotype. Isa Brown eggs had the highest values ($P \leq 0.026$) compared to the other two genotypes. The effect of housing system on eggshell percentage was recorded in the present study with higher values ($P \leq 0.006$) on litter than in cages. The effect of hen genotype on eggshell surface area was noticed. The values of Isa Brown eggs were significantly higher ($P \leq 0.001$) than those from Lohmann Brown and Hy-Line Silver Brown. The results are in agreement with Anderson et al. (2004) who reported different eggshell surface area of eggs from historic strains of single comb White Leghorn.

The results of cuticle deposition indicated that the laying hen genotype plays an important role in the deposition process (Table 1). A higher cuticle coverage ($P \leq 0.001$) was in eggs produced by Lohmann Brown compared to Isa Brown and Hy-Line Silver Brown. However, housing system did not significantly affect the cuticle coverage in

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the present study. Samiullah and Roberts (2014) reported a significantly higher cuticle deposition in cages versus free-range eggs. This might be explained by Kusuda et al. (2011) who concluded that the diversity in the structure of the cuticle layer may be linked to the environment of the nest, mainly humidity, which is hard to control in outdoor systems. Further investigation on the effect of genotype and housing management on cuticle deposition is needed because so-far available data are limited.

Our study indicated non-significant interactions of genotype and housing system. However, several studies indicated the effect of the interactions between genotype and housing system on eggshell quality parameters to be more important than the effect of individual factors (Singh et al. 2009; Zita et al. 2009; Tumova et al. 2011).

In conclusion, the results of the present study pointed out the important effect of laying hen genotype on egg weight, eggshell measurements, and cuticle deposition compared to the lower effect of housing systems. Selecting genotypes which provide higher shell quality characteristics and higher cuticle deposition ability is very important to maintain profitability and decrease bacterial penetration and egg spoilage.

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