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Interactions of housing system, genotype and eggshell quality and their relationship to egg safety

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Ph.D. Thesis Summary

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1. INTRODUCTION

Eggshell quality is one of the major problems facing poultry industry causing a huge impact on the profitability of egg production. The shell of the egg is very important to resist physical and pathogenic challenges from the external environment; also it is an important concern for consumers, as strong resistance to breaking and lack of shell defects are essential for protection against the penetration of pathogenic bacteria into eggs.

Mineralized eggshell is about (96%) calcium carbonate; the remaining components include the organic matrix (2%) as well as 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 (Hincke et al., 2008; Gautron et al., 2014). The structure of the eggshell and consequently eggshell characteristics are influenced by wide range of factors which combine to affect the final product. Housing systems, genotype and age are considered the most important factors (Ketta and Tůmová 2016). These factors are known to influence the eggshell quality characteristics, as well as, interactions between some of them could be more effective than individual factors.

According to housing systems, different eggshell quality parameters have been reported; heavier eggshells in cages than on deep litter (Pištěková et al., 2006). However, Tůmová et al. (2011) detected heavier eggshells on litter than in conventional and enriched cages. Eggshell thickness also varies according to housing systems. Comparing litter, free-range and cages housing systems, Pavlovski et al. (2001) detected thicker shells on litter eggs and thinner shells in free-range. Moreover, thicker eggshells on litter compared to cages were reported by Ledvinka et al. (2012). Eggshell strength differences were revealed by Englmaierová et al. (2014) who found stronger shells in cages than on litter. These contrast eggshell quality results could be either affected by hen genotype or different experimental conditions. Regarding to egg safety, Englmaierová et al. (2014) reported significant effect of housing systems on the total count of bacteria on the egg surface and the microbial contamination of *Enterococcus* and *Escherichia coli*. The lowest values were found in eggs from conventional cages while, the highest level of contamination was observed in eggs that were laid on litter.

Marked differences in eggshell quality follow from the particular breed, line and family of the laying hens. Tůmová et al. (2011) found the heaviest eggshells in Isa Brown in comparison with Hisex Brown or Moravia BSL. Differences between white and brown hybrids in eggshell thickness were described by Ledvinka et al. (2000) and Leyendecker et al. (2001) who found thicker shells within brown hybrids. Higher shell strength was revealed in white eggs layers in comparison with the brown ones (Ledvinka et al. 2000). Non-significant differences in shell strength were determined by Tůmová et al. (2007) in variable Dominant strains.

The eggshell characteristics might vary in different stages of laying hens age. The founding of Tůmová and Ledvinka (2009) indicated that, eggshell weight increased with hens age. The eggshell thickness also decreased with advancing age as declared by Bozkurt and Tekerli (2009). Similar trends were also found regarding to eggshell strength. Tůmová et al. (2014) detected a decreasing in eggshell strength in older hens (3.33 kg/cm^2) in comparison with the younger ones (3.60 kg/cm^2). Hence, the correct choice of housing systems will improve the eggshell characteristics and safety of eggs. Along with a genetically selected hen genotype for eggshell characteristics the economic losses related to damaged eggs will be reduced.

2. SCIENTIFIC HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

The eggshell quality traits play an important role concerning profitability as only eggs with an intact shell are considered for hatching or as table eggs. Therefore, if the eggshell quality parameters (mainly thickness and strength) are guaranteed, the industry could increase the number of eggs produced by each hen housed. The thickness of the eggshell as an important indicator for overall eggshell quality. Therefore, would eggs of different eggshell thickness affect other eggshell characteristics when eggs housed in different housing systems? The hypothesis was also set to compare the affectivity of housing system and genotype and the interaction between them on eggshell quality parameters and cuticle deposition. Using constant genotype, would the interaction of housing system, age and storage time have effect on the eggshell quality and egg safety?

2.2. Objectives

Regarding to the importance of eggshell mentioned above, it is necessary to study which factors might affect its characteristics. Furthermore, studying the possible interactions between those factors.

The aim of the study was to investigate the relationship between eggshell quality parameters in two different housing systems. In addition, to study the interaction of housing system and genotype on eggshell quality and cuticle deposition. Finally, to evaluate the effect of the housing system, age and their possible interactions on eggshell quality, microbial contamination and the penetration of microorganisms during different storage time.

3. MATERIALS AND METHODS

During the PhD study, four experiments were done. All the experiments were approved by the Ethics Committee of the Czech University of Life Sciences Prague and the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic.

3.1. Experiment 1

The study was designed to determine the relationship between eggshell thickness and other eggshell measurements in eggs produced in litter and enriched cages. The eggshell quality parameters were evaluated in 200 laying hens of ISA Brown at the age of 40-42 weeks. Laying hens were housed in enriched cages (100 hens, 750 cm^2/hen, 10 hens/cage) and in littered pens with wood shavings (100 hens, 9 hens/m^2, 10 hens/pen). The eggs were split into three categories differed in thickness: 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 metabolizable 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 Skřivan et al. (2015).

Eggs were analyzed every week three days in a row, and individually weighed, length and width of each egg were measured for egg shape index calculation (width/length × 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): Egg surface area = 4.67× (egg weight)$^{2/3}$. Eggshell index was calculated according to the followed equation: Eggshell index = (shell weight/shell surface) x 100 (Ahmed et al., 2005). Data were statistically analyzed 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.

3.2. Experiment 2

The study investigated the differences in the eggshell quality and the tibia measurements between Lohmann White and Czech Hens housed in conventional cages and on litter system.

Total number of 123 laying hens of Lohmann White and pure breed Czech Hen were housed in conventional cages Eurovent (72 hens, 550 cm^2/hen, 3 hens in a cage, 12 cages for genotype) and in six littered pens (60 hens, 7 hens/m^2, 10 hens/pen and 3 pens for each genotype). The experiment was carried out in the second half of laying cycle. Laying hens in both housing systems were fed commercial type of feed mixtures. The daily photoperiod consisted of 15 h light and 9 h darkness. Eggs for the egg shell quality assessment were collected in two weeks interval, two days in row, all eggs laid from each cage or litter pen and there were analyzed 300 eggs of Lohmann and 150 eggs of Czech Hen. Eggs were weighed, and the shell strength was determined by the shell-breaking method using a QC-SPA device (TSS York, UK). Eggshell weight was determined after drying. Eggshell thickness was evaluated by QCT shell thickness micrometer (TSS York, UK). Eggshell proportion was calculated from dried eggshell weight and egg weight.

Tibia characteristics were determined in 48 hens, 12 birds per a group, at 50 weeks of age. After slaughtering, both tibias were completely removed from the carcass. Weight, strength were measured in the right tibia. Tibia strength was measured by QC-SPA device (TSS York, UK) and thickness by micrometer QCT (TSS York, UK). Tibia Ca content was analyzed in the left tibia after ashing at 550 ^{o}C overnight using the method of AOAC 965.17 based on vanad-molybden reagent and spectrophotometry analysis on Solaar M6 apparatus (TJA Solutions, Cambridge, UK).

3.3. Experiment 3

The aim of the study was to compare the eggshell characteristics and cuticle deposition of Lohmann Brown, Hy-Line Silver Brown and Isa Brown housed in two different housing systems. The experiment was conducted on Lohmann Brown, Hy-Line Silver Brown and Isa Brown laying hens at the age of 40-56 weeks. Laying hens 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). Laying hens in both housing systems were fed identical commercial feed mixture with 15.37% crude protein, 11.58 MJ of metabolizable 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. During the experiment, eggs were collected in four weeks interval to be 660 eggs in total and divided into two groups; 330 eggs were used for analyzing of eggshell quality characteristics and the other 330 eggs were used to estimate cuticle deposition.

Freshly laid 330 eggs were individually weighed, length and width of each egg were measured for egg shape index calculation (width/length × 100). Eggshell strength was determined by the shell-breaking method using a QC-SPA device (TSS, England). Eggshell thickness was measured with a QCT shell thickness micro-meter (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): Egg surface area = 4.67× (egg weight)$^{2/3}$.

The total number of 330 eggs were used for cuticle estimation by a method of Roberts et al. (2013). Eggshells were individually soaked in MST cuticle blue stain (MST Technologies, Europe Ltd) for 1 min and rinsed in tap water 3 times to remove excess stain. The eggshell colour was measured using Konica Minolta hand-held spectrophotometer (CM-2600d) 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). The experiment data were evaluated with ANOVA, two-way analysis of variance using the GLM procedure of SAS (SAS, 2013). The model included the effects of genotype and housing system. A P-value of $P<0.05$ was considered significant for all measurements.

3.4. Experiment 4

The study was oriented to evaluate the effect of housing system, and age on eggshell quality, microbial contamination and micro-organisms penetration into the eggs of Isa Brown. The experiment was conducted with ISA Brown hens. Laying hens were housed in enriched cages (60 hens, 10 hens per cage, 750 cm^2 per hen) and in free range (60 hens, 9 hens per m^2) environments. The laying hens in the free range environment were placed in one deep-litter pen with wood shavings and with access to run. The daily photoperiod consisted of 15 h of light and 9 h of darkness. Laying hens were fed identical commercial feed mixtures N1 (with 18.7% crude protein and 11.5 MJ of metabolizable energy) from 20 to 40 weeks of age and N2 (with 15.3% crude protein and 11.4 of metabolizable energy) from 41 weeks of age. Feed and water were supplied *ad libitum*. The microclimate conditions were in accordance with the laying hen´s requirements (Skřivan et al., 2015).

Eggs were collected for three consecutive days during the 26th and 51st week to determine egg weight and eggshell quality. A total of 150 eggs were collected from each housing system and at each age (thus total of 600 eggs were analysed). The freshly laid eggs were individually weighed. The eggshell strength was measured using a destructive method that was performed with a QC - SPA apparatus (TSS, York, England). The eggshell thickness at the equatorial plane was evaluated using a QCT micrometre (TSS, York, England) after removing the inner and outer eggshell membranes. The eggshell weight was measured after drying at 50 °C for 2 h. The eggshell index was calculated as follows: shell weight/shell surface × 100 (Ahmed et al., 2005). The pore density was determined on the sharp end, blunt end and equator of each egg according to the method of Tůmová et al. (2011).

The eggs for the microbial contamination analyses were also collected during the 26th and 51st week of age, and 30 eggs from each housing system and each age were collected from the middle floor of the cages or from nests on the litter. The microbial analysis of the eggshell surface and egg content was performed with fresh eggs and eggs stored at 2, 7, 14 and 21 days. The numbers of *Escherichia coli* (EC), *Enterococcus* (ENT) and the total number of microorganisms (TNM) were recorded. Microbial analysis of the eggshell surface was performed according to Svobodová et al. (2015).

The data were statistically evaluated using the (GLM) procedure of SAS software (Statistical Analysis System, Version 9.1.3., 2003). The data for egg weight and eggshell quality characteristics were analysed with a two-way analysis of variance (ANOVA) with the housing system and age interactions, and the data for the microbial contamination of eggshells was evaluated by a three-way interaction analysis of variance (ANOVA) with the housing system, age and storage time interactions.

4. RESULTS AND DISCUSSION

The aims this thesis work were to evaluate the effect of housing system and genotype on the eggshell quality characteristics and cuticle deposition. Additionally, to study the relationship between the eggshell quality characteristics. Lastly, to estimate the eggshell microbial contamination and penetration of microorganisms into the egg content of fresh and stored eggs laid by hens of different age housed in two different housing systems.

Egg weight plays an important role as an indicator for most of the eggshell quality parameters. Ketta and Tůmová (2014) obtained heavier eggs ($P<0.001$) in enriched cages than those from litter. Similar observation ($P<0.001$) was recorded by Vlčková et al. (2018) between enriched cages and free-range systems. Moreover, the egg weight was affected by the interactions between the housing system and age (Vlčková et al., 2018). The heaviest eggs ($P<0.001$) were laid in free range at 51 weeks of age, and the lightest were detected in the same housing at 26 weeks which is in agreement with data Englmaierová et al. (2014).

The effect of housing system on eggshell thickness was not significant in the studies of Ketta and Tůmová (2018a, b). Similarly, Dong et al. (2017) and Yilmaz Dikmen et al. (2017) reported non-significant effect of housing systems on eggshell thickness. However, Ketta and Tůmová (2014) revealed thicker eggshells ($P<0.003$) on litter system compared to cages in both experimented genotypes which corresponded with the findings of Tůmová et al. (2011) who indicated thicker eggshells from laying hens kept on litter system compared with those from cages. Moreover, Vlčková et al. (2018) obtained thicker eggshells ($P<0.001$) in enriched cages compared to the free-range ones.

Regarding to eggshell strength, Ketta and Tůmová (2014) and Ketta and Tůmová (2018b) indicated a non-significant effect of housing system on eggshell strength. However, in the study of Ketta and Tůmová (2018a) and Vlčková et al. (2018) stronger eggs ($P<0.001$) were produced in cages compared to litter and free-range system. Similar observations were reported by Lichovníková and Zeman (2008) and Tůmová et al. (2011) who found higher eggshell strength in eggs from cages. The interactions between individual factors were also detected by Vlčková et al. (2018) where eggs with stronger ($P<0.01$) eggshells (46.1 g/cm^2) were laid by younger hens housed in enriched cages. Eggshell thickness and strength might be related to tibia breaking strength which is an important welfare problem for laying hens. Leyendecker et al. (2001) suggested that the eggshell stability and thickness seem to be negatively correlated with the bone strength. The effect of housing system on the tibia breaking strength was found by Ketta and Tůmová (2014). The tibia strength was higher (P<0.004) on litter system than in conventional cages which is in agreement with Lichovníková and Zeman (2008) who reported that hens kept in cages have weaker bones than those in alternative housing. Moreover, Tůmová et al. (2016) confirmed a higher tibia weight and strength in hens housed on litter compared to cages.

The eggshell percentage was affected by housing system in the study of Ketta and Tůmová (2018a) with higher values (P<0.001) in eggs from litter housing system compared to cages eggs. Similar results were found by Hidalgo et al. (2008) and Englmaierová et al. (2014).

The results of Ketta and Tůmová (2018b) confirmed the literature published data concerning the differences in eggshell quality according to different laying hen genotypes. Eggs produced by Lohman Brown hens were heavier (P<0.001) compared to those of Isa Brown and Hy-line Silver Brown. Moreover, comparing Lohmann White and Czech Hen, Ketta and Tůmová (2014) found higher egg weights (P<0.001) of eggs laid by Lohmann White than by Czech Hen. Similar observations were reported by Tůmová et al. (2011) who detected various egg weights from different hen genotypes. Contrary, non-significant effect of brown, white and tinted eggs laying hens on egg weight was reported by Tůmová et al. (2017).

The eggshell strength was not affect by hen genotypes in the studies of Ketta and Tůmová (2014) and Ketta and Tůmová (2018b). However, different experiments with also brown egg hybrids (Isa Brown, Hisex Brown, and Moravia BSL) indicated significantly stronger shells in Isa and Hisex Brown eggs (Tůmová et al. 2011; Ledvinka et al. 2012). In spite of the non-significant effect of genotype on eggshell strength, laying hen genotype significantly affected the eggshell thickness (Ketta and Tůmová 2014; 2018b). Thicker eggshells (P<0.003) of eggs of Lohmann White than Czech Hen were obtained by Ketta and Tůmová (2014). Moreover, Isa Brown produced the thickest eggshells (P<0.033) in comparison with Lohmann Brown and Hy-Line Silver Brown (Ketta and Tůmová 2018b). Non-significant effect of genotype on eggshell percentage was found by Ketta and Tůmová (2014). On the other hand, (Ketta and Tůmová 2018b) revealed that eggshell percentage and eggshell surface area were significantly affected by hen genotype, Isa Brown eggs had the highest values (P<0.001) compared to the other two genotypes. The eggs of Isa Brown hens were also longer (P<0.019) than those from Lohmann Brown and Hy-Line Silver Brown resulting in significantly higher egg shape index values.

Studying the effect of laying hen age on eggshell characteristics, Vlčková et al. (2018) obtained a significant interactions between housing system and age for egg weight ($P<0.001$), eggshell weight ($P<0.001$), eggshell strength ($P<0.01$) and shell index ($P<0.05$) were observed in the study. Eggs became heavier with advancing age in free-range system compared to cages.

Regarding to the egg safety, the important role of hen genotype on cuticle deposition was observed by Ketta and Tůmová (2018b). Higher cuticle coverage ($P<0.001$) was obtained in eggs produced by Lohmann Brown compared to Isa Brown and Hy-Line Silver Brown. However, housing system did not affect the egg cuticle deposition. Contrary, Samiullah et al. (2014) reported significantly higher cuticle deposition in cages versus free-range eggs. On the other hand, Vlčková et al. (2018) indicated that, the housing system play the key role regarding to eggshell contamination. A higher contamination ($P<0.001$) was found in free-range eggs compared to cages in all the monitored species of microorganisms. The results are in agreement with Belkot and Gondek (2014), who observed a lower number of aerobic bacteria in cages system compared to litter, free-range and the organic system. This led to a higher microbial penetration into free-range eggs.
Eggshell pores are considered as the pathway for microorganisms to penetrate into the egg content. The interactions between housing system and age were found for pores density by Vlčková et al. (2018). Higher numbers of eggshell pores ($P<0.001$) were detected in free-range eggs laid by older hens compared to enriched cages eggs laid by younger hens. Similarly, Tůmová et al. (2011) reported higher pore density in eggs from litter system compared to cages. However, numerically higher pores density in cages than on litter especially in the sharp end of the eggshell were detected by Ketta and Tůmová (2014).
Vlčková et al. (2018) also revealed a significant effect of storage time on eggshell microbial contamination. The number of *Escherichia coli* and *Enterococcus* decreased ($P<0.001$) with storage time, which corresponds with De Reu et al. (2006), who observed decreasing of aerobic bacteria and the total count of Gram-negative bacteria within 14 days of storage.

The study of Ketta and Tůmová (2018a) was more focused to estimate the relationship between eggshell characteristics of eggs produced in cages and on litter using Pearson's correlations coefficients. Significantly positive correlations between eggshell thickness and egg weight in both housing systems were found. In enriched cages, eggs were significantly ($P<0.05$) heavier than in litter system. Positive relationship ($P<0.001$) between eggshell thickness and eggshell weight were found by as well in the study. The results are in correspondence with the findings of Tůmová and Ledvinka (2009) and Molnár et al. (2016) who reported that the eggshell thickness was positively correlated with eggshell weight. A highly positive correlations (P<0.001) between eggshell thickness and eggshell strength were also obtained by Ketta and Tůmová (2018a).

The correlations between egg shape index and eggshell strength were found to be positive (P<0.01). Moreover, the Pearson's correlations coefficients between eggs produced in cages and on litter indicated different values especially for eggshell thickness (P<0.001), eggshell strength (P<0.001) and eggshell weight (P<0.001) (Ketta and Tůmová 2018a). A higher negative correlations (P<0.001) between eggshell percentage and egg weight was found on litter system compared to enriched cages. While, the correlations between eggshell thickness; eggshell weight and eggshell percentage were highly positive (P<0.001) on litter system. However, there are lack of published data concerning the correlations differences of eggshell characteristics regarding to different housing systems. Moreover, Ketta and Tůmová (2018a) reported an interactions of shell thickness category and housing system for eggshell percentage (P<0.001). 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.

To summarize, regarding to the eggshell quality characteristics, the results of this thesis work indicated the important effect of individual factors represented in laying hen genotype, age and housing system compared to the lower effect of their interactions. However, only laying hen genotype controlled the eggshell cuticle deposition. Housing system play the major role concerning the egg safety. Enriched cages are highly recommended to egg producers as it produce eggs with lower eggshell microbial contamination as well as lower microbial penetration into the egg content. The effect of eggshell thickness on overall eggshell quality characteristics was well noticed in the studies of this thesis. Therefore, it is of outmost importance for egg producers to keep laying hens genetically selected for higher eggshell thickness. However, further future studies concerning the relationship between eggshell thickness and other shell quality parameters is required to maintain the egg industry and a safe eggs and egg products.

5. SUMMARY

The aim of this dissertation work was to evaluate the effect of housing system, genotype and their possible interaction on eggshell quality characteristics and egg safety. To detect these differences, four experiments were carried out.

The first experiment was aimed to determine the relationship between eggshell thickness and other eggshell measurements in eggs produced in litter and enriched cages. To evaluate the previously mentioned relationship, 200 laying hens of ISA Brown at the age of 40-42 weeks were housed in enriched cages and in littered pens. The eggs were split into three categories differed in its thickness: thin, medium and thick shells. The eggshell parameters were measured including: egg weight, length and width of the egg, eggshell strength, eggshell thickness, eggshell percentage, egg surface area and eggshell index. The relationship between eggshell parameters was calculated separately in cages and on litter by estimating Pearson's correlation.

The second experiment investigated the differences in the eggshell quality and the tibia measurements between Lohmann White and Czech Hens housed in conventional cages and on litter system. A total number of 123 laying hens of Lohmann White and pure breed Czech Hen in the second half of laying cycle were housed in conventional cages and on littered pens. All eggs laid from each cage or litter pen and there were analyzed 300 eggs of Lohmann and 150 eggs of Czech Hen. For eggs weight, eggshell strength, eggshell weight and eggshell thickness. Tibia characteristics were determined in 48 hens, 12 birds per a group, at 50 weeks of age. After slaughtering, both tibias were completely removed from the carcass. Tibia weight, strength and thickness were measured.

The third experiment evaluated the eggshell characteristics and cuticle deposition of Lohmann Brown, Hy-Line Silver Brown and Isa Brown housed in enriched cages and on litter system. During the experiment, eggs were collected in four weeks interval to be 660 eggs in total and divided into two groups; 330 eggs were used for analyzing of eggshell quality characteristics including: egg weight, length, width, shape index, strength, thickness, eggshell weight, percentage and egg surface area. The remained 330 eggs were used to estimate cuticle deposition.

The fourth experiment was designed to study the effect of housing system, and age on eggshell quality, microbial contamination and micro-organisms penetration into the eggs of Isa Brown. Laying hens were housed in enriched cages and in free range environments. Total number of 150 eggs were collected for three consecutive days during the 26th and 51st week to determine egg weight, eggshell quality and pores density. Microbial contamination analyses were done in eggs also collected during the 26th and 51st week of age, from different housing system and age. The microbial analysis of the eggshell surface and egg content was performed with fresh eggs and eggs stored at 2, 7, 14 and 21 days. The numbers of *Escherichia coli* (EC), *Enterococcus* (ENT) and the total number of microorganisms (TNM) were recorded.

The studies of this thesis pointed out the significant effect of housing system on egg weight with heavier eggs in enriched cages than those from litter and free-range systems. The effect of age on egg weight was also recorded as the eggs became heavier with advancing age. The housing system had a significant impact on eggshell thickness and strength. Thicker and stronger eggshells were recorded from eggs laid on litter system compared to cages. This might be related to higher tibia strength on litter system than in conventional cages. The interactions between housing system and laying hen age were recorded also for eggshell strength with stronger eggshells were laid by younger hens housed in enriched cages. Moreover, Pearson's correlations coefficients were positive between most of the eggshell characteristics with higher values on litter than in cages.
The effect of laying hen genotype was well noticed in the studies. Eggs produced by Lohman Brown hens were heavier compared to those of Isa Brown and Hy-line Silver Brown. Moreover, higher egg weights of eggs laid by Lohmann White than by Czech Hen. Laying hen genotype significantly affected the eggshell thickness with thicker eggshells of eggs of Lohmann White than Czech Hen. Moreover, Isa Brown produced the thickest eggshells in comparison with Lohmann Brown and Hy-Line Silver Brown. The important role of hen genotype on cuticle deposition was observed. Higher cuticle coverage was obtained in eggs produced by Lohmann Brown compared to the other genotypes. However the effect of housing system did not affect the egg cuticle deposition. In the meaning of egg safety, the housing system was the key role regarding to eggshell contamination. A higher contamination was found in free-range eggs compared to cages in all the monitored species of microorganisms.

As indicated, maintaining the eggshell quality is more controlled by laying hen genotype and age compared to the lower effect of their interactions. Improving the eggshell thickness consequently improved overall eggshell quality parameters, therefore, it is highly recommended for egg producers to keep laying hens genetically selected for higher eggshell thickness. For keeping the produced eggs uncontaminated, higher attention should be paid to housing system types. Enriched cages are highly recommended to egg producers as it decrease the eggshell microbial contamination and penetration into the egg content.

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