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Effect of Housing System and Age of Laying Hens on Eggshell Quality, Microbial Contamination, and Penetration of Microorganisms into Eggs

Jana Vlčková[1,2]*, *Eva Tůmová*[2], *Mohamed Ketta*[2], *Michaela Englmaierová*[1], *Darina Chodová*[2]

[1]*Institute of Animal Science, Prague-Uhříněves, Czech Republic*

[2]*Czech University of Life Sciences Prague, Prague, Czech Republic*

**Corresponding author: vlckova.jana@vuzv.cz*

ABSTRACT

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Hens of the laying hybrid ISA Brown were used in the study with the objective to evaluate eggshell quality, microbial contamination of eggshells, and penetration of microorganisms into the egg content in different housing systems (enriched cage: 60 hens, 10 hens per cage, 750 cm^2 per hen vs free range: 60 hens, 9 hens per m^2) and at different hen ages (26 vs 51 weeks) during storage time (0, 2, 7, 14, and 21 days). A significant interaction between the housing system and age was observed in egg weight and most of eggshell quality measurements. However, microbial contamination and penetration were affected mostly by the housing system and storage time. The numbers of *Escherichia coli* ($P < 0.001$, 4.51 vs 2.75 log cfu/eggshell) and *Enterococcus* ($P < 0.001$, 2.56 vs 1.11 log cfu/eggshell), and the total number of microorganisms ($P < 0.001$, 5.04 vs. 3.65 log cfu/eggshell) were higher in free range eggs compared to enriched cage eggs, respectively. The counts of *Escherichia coli* ($P < 0.001$, 4.23 vs 2.91 log cfu/eggshell) and *Enterococcus* ($P < 0.001$, 2.31 vs 1.27 log cfu/eggshell) decreased with storage time. A positive correlation between the total number of pores and penetration of *Escherichia coli* in both housing systems was observed in the albumen. It can be concluded that the housing system and age of laying hens significantly affected eggshell quality. Microbial contamination presumably affects the penetration of microorganisms. The correlation between the number of pores and penetration is assumed to be affected by the microbial species.

Keywords: enriched cage; free range; egg safety; hen

In the commercial egg industry, the eggshell protects the egg from mechanical damage and contamination of the internal contents. Failure of the shell for any reason compromises the value of an egg as a food product. Egg producers must be aware of these factors because the economic consequences of shell failures are significant. At the time when the eggshell is formed, all of the investment of nutrients has already been made, and the loss of nutritional value potentially represents a total loss to the farmer (Hunton 2005).

There are many factors that affect the functional quality of the eggshell, mostly prior to when the egg is laid, such as the strain, the age of the bird,

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nutrition, stress, disease, and the housing system. As already mentioned, the housing system has a considerable effect on eggshell quality. However, the results of the effect of the housing systems on eggshell quality are ambiguous. Eggshell quality is characterized by many indicators, such as eggshell weight, specific weight, share, thickness, deformation or strength. Major economic losses for egg producers are associated with lower eggshell strength leading to eggshell breakage. Mertens et al. (2006) reported that shell strength was the greatest in aviary eggs and the weakest in free-range eggs. Inconsistent results explainable by structural differences of the eggshell are related to the interaction of the housing system, age, genotype, oviposition time, and mineral nutrition (Ketta and Tumova 2016).

Hen age is also one of the most important factors affecting shell quality. Very young birds with immature shell glands produce shell-less eggs or eggs with a thin eggshell (Ketta and Tumova 2016). Tumova et al. (2014) detected a decreased eggshell strength in older hens in comparison with younger ones. It is likely that these structural differences in eggshell formation may also affect pore density (Tumova et al. 2011).

The safety of egg production depends on eggshell contamination and the penetration of microorganisms into the egg. De Reu et al. (2006a) reported a significant higher average eggshell contamination by aerobic bacteria and the Gram-negative bacteria of eggs from alternative housing systems compared to conventional cages. Schwarz et al. (1999) found that the number of aerobic bacteria was higher in free-range eggs than in cage eggs. Jones et al. (2002) observed that the bacterial contamination of air cells, shells, and egg contents was more common in eggs from older hens than from younger ones.

Microorganisms on the egg surface can penetrate into the egg contents. The results of a study by De Reu et al. (2006b) showed that the most frequent percentage of eggshell penetration was by *Pseudomonas* sp. and *Alcaligenes* sp. followed by *Salmonella* Enteritidis in the eggshell. These microorganisms accounted for 60, 58, and 43% of the agar-filled egg penetration, respectively. De Reu et al. (2006b) and Messens et al. (2007) proved that higher eggshell contamination led to a greater possibility of microorganism penetration and egg content contamination, which may be related with a higher contamination of eggs in alternative housing systems. Some earlier studies observed the effect of quality of eggshells on microbial penetration. Sauter and Petersen (1974) determined that bacteria of the genus *Pseudomonas* were able to more readily penetrate into whole eggs of poor shell quality. However, De Reu et al. (2006b), who compared seven selected bacterial species, concluded that the weight of eggshell or eggshell thickness had no significant effect on penetration. The effect of the number of pores on the bacterial penetration was studied by Messens et al. (2005) and confirmed that a higher penetration was detected at the blunt pole of the egg. However, De Reu et al. (2006b) did not find a correlation between the number of pores and the bacterial eggshell penetration in aerobic bacteria and Gram-negative bacteria.

Contradictory data on the effect of the housing system, eggshell quality, and penetration of microorganisms into eggs need further research. It might be expected that there is an interaction between the housing system and the other factors. Therefore, the aim of this study was to evaluate the effect of the housing system, hen age, and their possible interactions on the eggshell quality, microbial contamination, and penetration of microorganisms into eggs during 21 days of storage at room temperature.

MATERIAL AND METHODS

The experiment was 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.

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 MJ of metabolizable energy) from 41 weeks of age.

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Feed and water were supplied *ad libitum*. The microclimate conditions were in accordance with the laying hen's requirements (Skrivan et al. 2015).

Eggs were collected for three consecutive days in weeks 26–51 to determine egg weight and eggshell quality. A total of 150 eggs were collected from each housing system and at each age (thus totally 600 eggs were analyzed). 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 Ltd., UK). The eggshell thickness at the equatorial plane was evaluated using a QCT micrometre (TSS Ltd.) 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). For the pore density determination, the shells were boiled in a 5% NaOH solution for 15 min to remove the shell membranes and then rinsed three times in distilled water. The rinsed eggshells were dried in an oven heated to 50°C. The inside surface of the shells was dyed with methylene blue. The dye solution was made by dissolving 0.5 g of 89% methylene blue crystals in 1 litre of 70% ethanol. The pores appeared as blue dots on the outside surface due to capillary action. The pore density was determined on the sharp end, blunt end, and equator of each egg. The average number of pores from three parts multiplied by the area of the egg was calculated.

The eggs for the microbial contamination analyses were also collected in weeks 26–51 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 analyses of the eggshell surface and the egg content were performed with fresh eggs and stored eggs at 2, 7, 14, and 21 days. The eggs were stored at room temperature (20–22°C) and a relative humidity of 55–60% on clean plastic egg cartons. A total of 120 eggs were analyzed. 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 Svobodova et al. (2015). The eggs were sampled by hand (wearing clean gloves) and placed on a clean plastic egg carton. To determine shell contamination, the eggs were placed into sterile plastic bags with 10 ml of sterile saline peptone (9 g sodium chloride, 1 g peptone, and 1000 ml distilled water) in which they were thoroughly rinsed for 2 min. A dilution series for each egg was produced by adding 1 ml of the solution (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). The determination of the egg content contamination was based on disinfection of the eggshell surface with ethanol and aseptic removal of the eggshell membrane and thin albumen. The microorganism analysis was conducted with standard agar methods. The number of EC was monitored using Mac-Conkey agar, the number of ENT using Slanetz Bartley agar, and TNM using Standard Plate Count agar (all Oxoid, UK). Plates with Mac-Conkey agar and Slanetz Bartley agar were then incubated for 48 h in an incubator at 37°C. The Standard Plate Count agar was incubated for 120 h in an incubator at 30°C. Typical colony forming units (cfu) on the eggshell were counted on a Petri dish after incubation. The percentages of times at which the microorganisms penetrated into the egg content were calculated afterwards.

The data were statistically evaluated using the General Linear Models (GLM) procedure of the SAS software (Statistical Analysis System, Version 9.1.3., 2003). The data for egg weight and eggshell quality characteristics were analyzed with a two-way analysis of variance (ANOVA) with the housing system and age interactions, and the data for the microbial contamination of eggshells were evaluated by a three-way interaction analysis of variance (ANOVA) with the housing system, age, and storage time interactions. All of the differences were considered significant at $P < 0.05$. The results in the tables were presented as the means and standard error of the means (SEM). The relationship between the total number of pores and penetration of the microorganisms was evaluated by estimating Pearson's correlation coefficient.

RESULTS

Egg weight and eggshell quality characteristics are provided in Table 1. The egg weight was affected by a two-way interaction ($P < 0.001$) between the housing system and age. The heaviest eggs were laid in free range at 51 weeks of age, and the lightest were detected in the same housing at 26 weeks. The egg weight was significantly higher in enriched cages compared to free range and increased with advancing age ($P < 0.001$).

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Regarding to eggshell quality characteristics, the two-way interaction between the housing system and age ($P < 0.001$) was observed in the eggshell strength. The strongest eggshells were found in eggs in younger hens in the enriched cage, whereas the weakest occurred in the free range and in younger hens. Eggs with significantly stronger eggshells (46.1 g/cm^2) were laid in younger hens housed in enriched cages. The eggshell thickness was only affected by the housing system ($P < 0.001$). The significant interaction between the housing system and age in the eggshell weight showed that enriched cage eggshell weight was similar in eggs from young and old hens, whereas in free range, the eggshell weight was higher at 51 weeks. Significantly heavier eggshells were observed in eggs laid in cages compared to free range and in eggs from 51-week-old hens. A higher number of pores ($P < 0.001$) occurred in free range eggs and in older hens ($P < 0.001$).

Table 2 provides the results of the microbial contamination of eggs during storage. The contamination by EC was affected by a two-way interaction between the housing system and storage time ($P < 0.001$). According to the housing system, the EC number in free range was by approximately 1.76 log cfu/eggshell higher compared to enriched cages ($P < 0.001$). The counts of EC decreased during storage ($P < 0.001$) and were approximately 1.32 log cfu/eggshell. The contamination of ENT was significantly higher in free range in comparison with the enriched cage and was not affected by age. Regarding storage time, the counts of ENT ($P < 0.001$) were the highest on the second day. The total number of microorganisms was significantly affected by the interaction between age and storage time, and the housing system higher values were in free range.

The penetration of microorganisms into the eggshell membrane and albumen are shown in Table 3. No considerable differences in the penetration of EC into the eggshell membrane between cage and free range were found. However, the penetration of ENT through the eggshell membrane differed between the housing systems. ENT penetrated the eggshell membranes in eggs from cages only in young hens during the second day of storage, whereas in free range and at the same age this occurred on the second and seventh day of storage. In older free-range hens, penetration was observed on days 14 and 21 of storage. Regarding the housing system, a higher penetration of TNM on the eggshell membrane was recorded in free range eggs compared to enriched cages; however, the influence of age and storage time was not evident. In the albumen, more frequent penetration in free range was observed for EC and TNM; however, the effect of age and storage

Table 1. Results of eggshell quality characteristics

Characteristics	Item	Age (weeks)	Egg weight (g)	Eggshell strength (g/cm^2)	Eggshell thickness (μm)	Eggshell weight (g)	Shell index (%)	Total number of pores
Housing system	enriched cage		61.0^a	46.1^a	349^a	6.17^a	8.54^a	6958^b
	free range		59.0^b	38.9^b	315^b	5.27^b	7.82^b	7454^a
Age (weeks)	26		57.5^b	43.1^a	331	5.58^b	8.12	6906^b
	51		62.5^a	41.9^b	333	5.86^a	8.24	7507^a
	enriched cage	26	60.3^b	47.5^a	347	6.15^a	8.70^a	6632
		51	61.8^a	44.6^b	352	6.19^a	8.39^{ab}	7285
	free range	26	54.8^c	38.7^c	315	5.01^c	7.54^b	7180
		51	63.2^a	39.1^c	315	5.52^b	8.10^{ab}	7728
SEM			0.214	0.340	1	0.028	0.108	39.49
***P*-value**								
Housing system			***	***	***	***	***	***
Age			***	*	ns	***	ns	***
Housing system × age			***	**	ns	***	*	ns

results of the variance analysis are indicated as significant (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) or not (ns)

$^{a–c}$statistically significant differences in columns are indicated by different superscripts

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Table 2. Results of eggshell microbial contamination

Characteristics	Item	Storage time (days)	Bacterial strain log (cfu/eggshell)		
			EC	ENT	TNM
Housing system	enriched cage		2.75[b]	1.11[b]	3.65[b]
	free range		4.51[a]	2.56[a]	5.04[a]
Age (weeks)	26		3.62	2.02	4.38
	51		3.64	1.64	4.31
Storage time (days)	0		4.23[a]	2.31[ab]	4.68
	2		3.89[ab]	2.98[a]	4.53
	7		3.58[b]	1.41[b]	4.04
	14		3.55[b]	1.20[b]	4.19
	21		2.91[c]	1.27[b]	4.27
Enriched cage	26 weeks	0	3.63	1.16	3.51
		2	2.50	2.89	4.01
		7	2.96	1.26	3.89
		14	3.15	0.34	3.70
		21	1.21	0	3.16
	51 weeks	0	3.48	1.60	4.43
		2	2.96	2.01	3.60
		7	2.78	0.33	3.41
		14	2.85	1.15	3.43
		21	2.02	0.33	3.23
Free range	26 weeks	0	4.99	3.31	5.50
		2	5.06	3.61	5.66
		7	4.53	3.13	5.00
		14	4.16	2.03	4.93
		21	4.05	2.50	4.42
	51 weeks	0	4.87	3.17	5.24
		2	4.97	3.56	4.86
		7	4.04	0.87	4.03
		14	4.13	1.21	4.72
		21	4.37	2.25	5.95
SEM			0.083	0.116	0.083
***P*-value**					
Housing system			***	***	***
Age			ns	ns	ns
Storage time			***	***	ns
Housing system × age			ns	ns	ns
Housing system × storage time			**	ns	ns
Age × storage time			ns	ns	**
Housing system × age × storage time			ns	ns	ns

cfu = colony forming units, EC = *Escherichia coli*, ENT = *Enterococcus*, TNM = total number of microorganisms
results of the variance analysis are indicated as significant (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$) or not (ns)
[a–c]statistically significant differences in columns are indicated by different superscripts

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Table 3. Results of microbial penetration into the egg content

Housing system	Age (weeks)	Storage time (days)	Penetration (%)					
			eggshell membrane			albumen		
			EC	ENT	TNM	EC	ENT	TNM
Enriched cage	26	0	–	–	0.56	–	–	0.56
		2	1.11	0.56	0.56	–	–	–
		7	–	–	2.22	0.56	–	1.67
		14	0.56	–	1.67	–	–	1.67
		21	–	–	1.11	0.56	–	1.11
	51	0	–	–	1.11	–	–	0.56
		2	0.56	–	1.11	0.56	–	1.11
		7	0.56	–	1.67	–	–	–
		14	–	–	1.67	–	–	1.67
		21	1.11	–	1.11	–	–	0.56
Free range	26	0	–	–	2.22	–	–	1.67
		2	–	0.56	1.11	–	–	1.67
		7	–	0.56	2.22	0.56	–	2.22
		14	1.11	–	2.78	1.11	–	1.11
		21	0.56	–	2.22	–	–	0.56
	51	0	–	–	0.56	–	–	2.22
		2	1.11	–	3.33	0.56	0.56	1.11
		7	–	–	0.56	–	–	0.56
		14	2.22	1.67	1.11	1.67	1.67	1.11
		21	0.56	–	1.11	0.56	–	1.11

EC = *Escherichia coli*, ENT = *Enterococcus*, TNM = total number of microorganisms

time was not detected. ENT penetrated into the albumen only in free range eggs from older hens on the 2nd and 14th day of storage.

Correlations between the total number of pores and the penetration of microorganisms into the eggs are presented in Table 4. The results show a negligible relationship between the number of pores and the penetration of microorganisms through the eggshell membrane and into the albumen. Significant penetration was only observed in EC in both housing systems.

Table 4. Correlation between the total number of pores and the penetration of microorganisms

		Enriched cage	Free range
		total number of pores	
Penetration in eggshell membrane	EC	0.078	0.032
	ENT	0.178	0.017
	TNM	0.019	0.117
Penetration in albumen	EC	0.316**	0.240*
	ENT	–	0.121
	TNM	0.048	0.127

EC = *Escherichia coli*, ENT = *Enterococcus*, TNM = total number of microorganisms

Pearson's correlation coefficients are indicated as significant (*$P \leq 0.05$, **$P \leq 0.01$, *** $P \leq 0.001$)

DISCUSSION

A significant interaction between the housing system and age in egg weight was found in this study. In cages, the egg weight was increased with age within 1 g, whereas in free range, the weight increased to almost 9 g. These results are in correspondence with Van Den Brand et al. (2004), who also detected an interaction of age and the housing system, and the free range layers had eggs with lower weight than the cage layers at the beginning of the experiment; however, the egg weight in eggs from free range increased faster after 59 weeks and was greater than the egg weight

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in the cage. With respect to the housing system, heavier eggs were produced in enriched cage than produced from free range. Our results are in accordance with studies by Lewko and Gornowicz (2011), who also found heavier eggs in cages in comparison with free range. However, Hidalgo et al. (2008) reported that free range layers produced heavier eggs compared to other systems. In the literature, the results of egg weight in different housing systems are quite variable. These differences are probably caused by variable conditions such as genotype and feeding, among others. In agreement with Van Den Brand et al. (2004), the egg weight increased with advancing age.

All of the monitored characteristics of eggshell quality were influenced by the housing system. In the present study, stronger and thicker eggshells were laid by hens kept in cages. There was a two-way interaction between the housing system and age-affected eggshell strength and eggshell weight; however, in eggshell thickness, a significant effect was observed only in the housing system. Shell strength, one of the most important egg external quality parameters, is usually dependent on eggshell proportion and thickness. Our results were also confirmed in the study by Tumova et al. (2011) revealing stronger eggshells produced in the cage system compared with litter. Also Lichovnikova and Zeman (2008) reported higher eggshell strength in eggs from cages. The shells from eggs produced in cages seem to have ultrastructural features which support the eggshell strength. The rates of calcium deposition in shells of eggs produced in the two systems are possibly different (Tumova et al. 2011). Lichovnikova and Zeman (2008) showed that calcium content in the shell and calcium intake were higher in cages than on litter. Structural differences in the eggshell formation according to a housing system may be the result of variable pore density in eggs from cages and litter.

However, contrary to our results, Van Den Brand et al. (2004) recorded greater eggshell strength and thickness in free range eggs. Mertens et al. (2006) evaluated conventional cage, enriched cage, aviaries, and free range and found the greatest strength of the eggshell in aviary eggs, whereas the weakest was found in free range eggs. Differences in eggshell physical parameters are assumed to be related to eggshell microstructure. Differences in the eggshell structure might be indicated by the eggshell index. In the present study, the eggshell index was affected by the interaction between the housing system and age. The interaction showed differences between the housing systems at 26 weeks, whereas the measurement did not vary in older hens. Ahmed et al. (2005) noted that the eggshell index expresses the size of the crystals and the compactness of the eggshell. Smaller crystals in the eggshell are more compact and increase the strength of the eggshell. Structural differences can be associated with decreasing eggshell strength with age; however, the eggshell thickness was not influenced, which corresponds with Rodriguez-Navarro et al. (2002). The authors reported a weaker correlation between eggshell strength and thickness or weight in young hens than older ones. These changes could also be responsible for the decline of shell strength because the components of the organic matrix are involved in the control of shell mineralization and crystal orientation, and they contribute to its organization and therefore to the mechanical properties of the shell (Nys et al. 1999).

In this study a higher porosity was detected in free range eggs compared to enriched cage eggs. Similarly, Tumova et al. (2011) observed differences in the pore density between cages and litter in the equatorial area, and a higher number of pores were detected in eggs from litter. This parameter was in our study also influenced by age, with the highest values in 51-week-old hens; this was in accordance with Messens et al. (2005), who detected the highest porosity of the eggshell in the middle of the laying period. Additionally, these results may be explained by structural differences in the eggshell formation according to the housing system.

A significantly higher contamination of eggshells was found in free range compared to cage eggs in all of the monitored species of microorganisms. Our results are in accordance with a study by Belkot and Gondek (2014) who compared the microbial contamination of eggs from four different housing systems and observed a lower number of aerobic bacteria in the cage system compared to litter, free range, and the organic system. Vucemilo et al. (2010) showed that in terms of cleanliness, the cage is the most suitable system. Generally, a higher contamination of eggs by microorganisms is probably related to cleanliness (Singh et al. 2009). In alternative systems, birds move freely in their environment, and a significant amount of dust that

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originates from litter is created, which results in air contamination by microorganisms and endotoxins (Wathes 1994). In a study by De Reu et al. (2005a), the total count of aerobic bacteria in the air of poultry houses proved to be positively correlated with the initial bacterial eggshell contamination in the house. In our study, the microbial contamination of the eggshell was not affected by the age of the laying hens. However, Huneau-Salaun et al. (2010) detected that eggshell contamination increased significantly with the age of the laying hens in both flocks in cages and alternative systems. According to Mallet et al. (2003), contamination decreased with the age of hens kept in conventional and in furnished cages, but the authors attributed this decrease to a seasonal effect. However, a study by Kretzshmar-McCluskey et al. (2009) described that the microflora load on the shell increased with the age of hens.

According to the results of the present study, the microbial contamination of eggshells was also affected by storage time. The number of EC and ENT significantly decreased with time of storage, which corresponds with De Reu et al. (2005b) who observed that the total count of aerobic bacteria and the total count of Gram-negative bacteria significantly decreased within 14 days of storage time (from 4.04 to 3.23 log cfu/eggshell).

In our experiment the penetration of EC, ENT, and TNM was mainly affected by the housing system. A higher microbial penetration in the eggs from free range is assumed to be significantly affected by higher microbial contamination of the eggshells, and this assumption corresponds with Messens et al. (2007). Likewise, De Reu et al. (2007) detected a higher penetration into the egg content in eggs from an alternative housing system (2.3%) compared to eggs laid in an enriched cage (1.9%). In contrast to the housing system, the effect of age on the microbial penetration was not observed. However, Nascimento et al. (1992) reported an increasing eggshell penetration from 12.9 to 25% for *Salmonella* Enteritidis with advancing age. De Reu et al. (2006a) showed almost constant bacterial eggshell penetration during the laying period. Additionally, in this work the storage time did not significantly affect the microbial penetration. De Reu et al. (2006b) studied the influence of the storage time on the penetration of various bacterial species. Independent of the selected strain, the authors found that the eggshell penetration was observed most frequently at approximately 4–5 days. At day 6 and day 14, total eggshell penetration was up to 80% and more than 95%, respectively. The penetration of microorganisms can be affected by different factors such as eggshell quality, pore density, and others. For example, Sauter and Petersen (1974) observed that *Salmonella* more likely penetrated eggs with lower specific gravity and hence thinner shells. However, Messens et al. (2005) did not find a relationship between thickness and penetration of *Salmonella* Enteritidis. The pores of the eggshell can be the area of microbial penetration. In the present study, only a positive correlation between the number of pores and penetration was observed in EC. Board and Halls (1973) also found a correlation between the porosity and bacterial penetration. However, De Reu et al. (2006b) showed no significant relationship between the area of the eggshell, shell thickness, and the number of pores and bacterial eggshell penetration. From these contradictory results it is possible to assume that penetration may also be influenced by the species of bacteria and its activity. For instance, some types of microorganisms probably penetrate more easily than others, which was suggested by the study of De Reu et al. (2006b), in which *Pseudomonas* sp., *Alcaligenes* sp., and *Salmonella* Enteritidis penetrated most frequently compared to *Staphylococcus, Acinetobacter, Serratia,* and *Carnobacterium*.

CONCLUSION

The results of this study show the impact of the housing system and age, including their interaction, on egg weight and eggshell quality characteristics. A higher microbial contamination of the eggshell was detected in free range eggs. However, hen age had a minor effect on contamination. During the eggs storage, the number of EC and ENT gradually decreased. The penetration of bacteria into the egg content was probably related to the number of microorganisms on an eggshell surface. In addition, the positive correlation between the number of pores and penetration of EC into the albumen was observed in both housing systems. The results indicate that a relationship may exist between the quality of the eggshell and the penetration of selected species of bacteria into the egg.

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