Effect of genotype, age of hens and *K/k* allele on eggshell quality

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ABSTRACT: We assessed the influence of the particular genotype, age of layers, feather growth-rate gene, and their mutual interactions on selected indicators of eggshell quality in six groups of hens of the laying type Dominant. The following genotypes were examined in the experiment: Barred Plymouth Rock, Dominant BPR 951 (K) strain, slow-feathering; Barred Plymouth Rock, Dominant BPR 901 (k) strain, fast-feathering; Blue Plymouth Rock, Dominant BLPR 954 (K) strain, slow-feathering; Blue Plymouth Rock, Dominant BLPR 894 (k) strain, fast-feathering; crossbreds of the above strains in the F_1 generation Dominant D 107 blue (K), slow-feathering and Dominant D 107 blue (k), fast-feathering. The layers were fed a feed mixture NP1 (16.64 % CP) from the 20th week of age and a feed mixture NP2 (15.02% CP) from the 42nd week. Husbandry conditions met the regular requirements of laying hens. Egg production and live weight of hens were monitored for the duration of the experiment (12 months). Eggshell quality was examined at the layers' age of 27, 35 and 56 weeks. The average hen-day egg production for the duration of the experiment (12 months) was not significantly influenced by the particular genotype or the feather growth-rate gene. The varying representation of the feather growth-rate gene significantly ($P \leq 0.001$) influenced the live weight; similarly, the relationship between the genotype and the representation of *K/k* alleles was significant. The average egg weight was influenced statistically significantly ($P \leq 0.001$) by the age of hens, their genotype ($P \leq 0.05$), feather growth-rate gene ($P \leq 0.001$), and the relationship between the age and genotype ($P \leq 0.001$). The age of hens, genotype, and the interaction of these two factors affected the egg shape index, as did the incidence of the feather growth-rate gene within the population (with a statistical significance of $P \leq 0.001$). The age, genotype and the feather growth-rate gene incidence within the population also significantly affected the eggshell quality indicators. In the eggshell to egg ratio, eggshell thickness and strength, an interaction was determined between the age of hens and their particular genotype. The eggshell colour was also significantly ($P \leq 0.001$) affected by hens' age, genotype ($P \leq 0.001$), as well as by the feather growth-rate gene ($P \leq 0.001$). No significant interaction between the age and the genotype was found for this indicator.

Keywords: hens; laying type; age; genotype; *K/k* allele; eggshell quality

Current breeding programmes on numerous farms apply adaptation genetics with respect to interactions between the genotype and the environment in order to select hybrids with favourable egg-production parameters as well as technological qualities of eggs even under variable farming condi-

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tions. Such breeding programmes aim to produce layers with vitality, feathering (colour diversity), preferred eggshell colour, and suitability for several production cycles befitting the increasingly popular free-range farms as well as the needs of small farmers (Tyller and Holoubek, 2003).

Eggshell quality is one of the most important egg parameters and one of the indicators subjected to long-term monitoring in the breeding of layer hens. Eggs of poor shell quality cause significant losses to producers of consumer eggs. Nonstandard eggs usually account for 3 to 12% of the total yield (Jelínek, 1996; Ledvinka et al., 2000). Eggshell quality is most often expressed as the proportion of the eggshell in egg weight, and by thickness and strength factors. It is undisputable that the main biological factors affecting eggshell quality are internal factors such as genetics, hen's age, production cycle stage, egg weight, intensity and persistence of production, time of laying, combined with external factors such as the nutritional level, farm system and microclimatic parameters.

Marked differences in eggshell quality follow from the particular breed, line and family of the layer (Buss and Guyer, 1982). The total eggshell weight is directly proportionate to the egg size and eggshell thickness, with a high level of correlation between the thickness and strength of the eggshell (0.92–0.97) depending on the particular genotype (Harms et al., 1990). Zhang et al. (2005) reported heritability coefficients 0.64, 0.34 and 0.24 for eggshell weight, thickness and strength, respectively.

The layers' age is one of the factors exerting a significant influence on eggshell quality. Egg weight tends to increase with the age of the layer, as does the incidence of misshaped eggs, resulting in decreased eggshell thickness and decreased eggshell to egg ratio (Hamilton et al., 1979). Similar conclusions about decreasing eggshell thickness and eggshell to egg ratio were reported by Hamilton (1982), Roland (1984), and Doyon et al. (1985). However, Yannakopoulos and Tserveni-Gousi (1987) reported greater eggshell thickness in older layers. Eggshell quality is also strongly influenced by production intensity. There is usually a negative correlation between production intensity and eggshell quality (Grunder et al., 2008). Eggshell quality can also be a function of the time of laying during the day. Eggs laid in the afternoon tend to have the better quality of eggshell compared to eggs laid in the morning (Pavlovski et al., 2000; Tůmová et al., 2007; Tůmová and Ledvinka, 2009). On the other hand, Koga et al. (1982) stated that the highest incidence of eggs with poor quality shells was between 3 p.m. and 9 p.m. No definite impact of the time of laying on the eggshell weight was found by Aksoy et al. (2001).

Varying representation of the feather growth-rate gene within the genotype can also influence the eggshell quality. Mérat et al. (1992) observed a positive correlation between the feather growth rate and the eggshell thickness. Arent et al. (1997) reported inconclusive differences in the average eggshell weight in favour of slow-feathering Rhode Island Red hens, however, with the eggshell strength non-significantly higher in the fast-feathering subpopulation.

The objective of the present paper is to evaluate the influence of genotype, age of layers and feather growth-rate gene, and of the interactions of the above factors on eggshell quality.

MATERIAL AND METHODS

The experiment was conducted in the laying hall of a farm for layer hens over a period of 12 months. A total of 450 pullets were divided at the age of 16 weeks into six groups of 75 chickens according to the genotype and feather growth-rate gene.

The following genotypes were used in the experiment: Barred Plymouth Rock, Dominant BPR 951 (K) strain, slow-feathering; Barred Plymouth Rock, Dominant BPR 901 (k) strain, fast-feathering; Blue Plymouth Rock, Dominant BLPR 954 (K) strain, slow-feathering; Blue Plymouth Rock, Dominant BLPR 894 (k) strain, fast-feathering; a crossbred of the above strains in the F_1 generation Dominant D 107 blue (K), slow-feathering; and a crossbred of the above strains in the F_1 generation Dominant D 107 blue (k), fast-feathering (Table 1). The hens were housed in individual cages in a traditional three-deck battery system. The husbandry conditions corresponded to the regular requirements of poultry farming using battery cages. The hens were fed a feed mixture NP1 for hens in the first production cycle from the 20th week of age and a feed mixture NP2 from the 42nd week of age. The layers had *ad libitum* access to both feed and water. The feed mixture compositions and nutrient contents are listed in Table 2.

At 20 weeks of age, the birds were provided with 14 h of light, extended gradually to 16 h at 24 weeks of age. The average light intensity was 10 lx at the level of the middle battery deck.

Table 1. Scheme of the experiment

Group	Genotype		Number of hens	Number of eggs
1	Barred Plymouth Rock (BPR)	D 951 (K)	75	675
2		D 901 (k)	75	675
3	Blue Plymouth Rock (BLPR)	D 594 (K)	75	675
4		D 894 (k)	75	675
5	F_1 hybrid (F_1)	D 107 (K)	75	675
6		D 107 (k)	75	675

Eggs were collected for the purpose of determining shell quality parameters at the ages of 27, 35 and 56 weeks. The eggs were always collected at these ages on four consecutive days. For each analysis 225 eggs were collected from each group, i.e. 675 eggs at three analytic dates. Each analysis was performed for 1350 eggs from six groups of hens, i.e. 4050 eggs for the entire experiment.

The hen-day egg production and hens' live weight were monitored during the entire experiment. The following qualitative parameters were monitored for each group: average egg weight, egg shape index, eggshell to egg ratio, eggshell thickness, strength and colour. Eggshell strength was determined by the eggshell strength analyser QC-SPA manufactured by TSS England. Eggshell colour was evalu-

Table 2. Composition of feed mixtures

Component (%)	NP1	NP2
Wheat	36.45	46.00
Wheat bran	–	3.00
Wheat	–	5.00
Barley	3.00	–
Maize	27.50	22.30
Maize gluten	0.60	–
Soybean meal	20.10	13.00
Vegetable oil	2.10	0.30
Natural rock salt	0.25	0.30
VPN 306	0.30	0.30
Premix Lysine 100	0.10	0.10
Methionine premix	0.15	0.10
Dicalcium phosphate	1.40	1.10
Calcium carbonate	7.90	8.40
Sodium bicarbonate	0.15	0.10
Analyzed content of nutrients		
Crude protein (%)	16.64	15.02
Metabolizable energy (MJ)	11.50	11.09
Methionine (%)	0.41	0.33
Lysine (%)	0.88	0.71
Ca (%)	3.40	3.51
P total (%)	0.64	0.59

ated by an objective photometric method using a QCR reflectometer, also by TSS England, operating on the principle of determining the percentage of the light reflected by the eggshell surface within the interval from 0 to 100 %. Higher values corresponded to lighter eggshell shades.

The results for particular parameters were then used in a multi-parameter analysis of interactions among the genotype, age of hens and feather growth-rate gene. The statistical analysis was processed by the computer application SAS 9.2 (SAS Institute Inc., 2008).

RESULTS AND DISCUSSION

Table 3 shows that the average hen-day egg production during the laying experimental period was not significantly influenced by the particular genotype, different representation of the *K/k* alleles of the feather growth-rate gene within the population, or by the relationship between the genotype and the feather growth-rate gene.

The average live weight was significantly ($P \leq 0.001$) influenced by the feather growth-rate gene. There was also a statistically significant ($P \leq 0.001$) interaction between the genotype and the representation of the *K/k* alleles. However, there were no significant differences between the particular genotypes.

As demonstrated in Table 4, the average egg weight was significantly ($P \leq 0.001$) influenced by the age of hens and particular genotype ($P \leq 0.05$), with a statistically significant ($P \leq 0.001$) interaction between the age of hens and the genotype. Significant differences ($P \leq 0.001$) were found out between the fast- and slow-feathering strains of Barred Plymouth Rock, Blue Plymouth Rock hens and the fast- and slow-feathering hens of the F_1 generation. This fact demonstrates the influence of the feather growth-rate gene on egg weight.

At the ages of 35 and 56 weeks, the layers of the fast-feathering strain of Blue Plymouth Rock hens showed the highest egg weight. The table shows that the average egg weight for the particular groups of birds demonstrably ($P \leq 0.001$) increased with age, in agreement with numerous other sources (Rizzi and Chiericato, 2005; Johnston and Gous, 2007; Odabasi et al., 2007; Tůmová and Ledvinka, 2009; Zita et al., 2009). The demonstrated significant influence ($P \leq 0.05$) of the genotype on egg weight corresponds with Heil and Hartman (1997), Tůmová et al. (2007). The above authors frequently pointed out that the egg weight changes in hens of the same genotype in different periods, even under constant husbandry conditions, further supporting the fact that egg weight is affected by a range of factors. The ascertained influence of different representation of the feather growth-rate gene within the population also corresponds with the conclusions of other authors (Mérat et al., 1992; Arent et al., 1997). The results of the present study suggest the same conclusion, when for example in the case of Barred Plymouth Rock hens at the age of 27 weeks, the highest egg weight was recorded for the slow-feathering strain, while at the age of 56 weeks, the same was recorded for the fast-feathering strain. The same situation was observed in the F_1 crossbreds, when the slow-feathering hens demonstrated the highest egg weight at the age of 27 weeks, but much lower at the age of 56 weeks (60.91 g as opposed to 64.26 g). In the case of Blue Plymouth Rocks, the fast-feathering hens demonstrated significantly ($P \leq 0.001$) higher egg weight during the entire duration of the experiment.

The egg shape index (Table 4) was also significantly ($P \leq 0.001$) influenced by the age of birds, along with the particular genotype ($P \leq 0.001$) and

Table 3. The average performance of laying hens for the entire monitored period (12 months)

Parameter	Genotype						Significance		
	BPR		BLPR		F_1		genotype	*K/k* allele	genotype × *K/k* allele
	K	k	K	k	K	k			
Hen day egg production (%)	66.00	77.42	71.92	70.08	75.75	76.75	NS	NS	NS
Live weight (kg)	1.95	1.96	1.99	1.83	1.93	1.90	NS	***	***

NS = non-significant; ***$P \leq 0.001$; K = slow-feathering strains, k = fast-feathering strains

Table 4. Summary results of some parameters of the eggshell quality

Parameter	Age (weeks)	Genotype						Significance						
		BPR		BLPR		F_1		age	genotype	*K/k* allele	age × genotype	age × *K/k* allele	genotype × *K/k* allele	age genotype × *K/k* allele
		K	k	K	k	K	k							
Egg weight (g)	27	54.58	53.75	52.15	55.14	54.07	53.14	***	*	***	***	***	**	NS
	35	62.15	62.47	60.75	63.42	62.17	63.15							
	56	63.36	64.53	62.87	64.91	60.91	64.26							
Egg shape index (%)	27	77.54	77.27	77.27	75.99	77.38	76.21	***	*	***	*	NS	***	NS
	35	75.49	76.36	75.88	74.09	76.12	75.32							
	56	74.29	74.85	75.57	74.66	75.49	74.84							
Proportion of eggshell (%)	27	10.97	10.79	10.93	11.41	11.35	11.63	***	***	***	***	NS	***	***
	35	10.62	10.62	10.91	11.04	10.83	11.37							
	56	10.87	10.89	10.98	11.45	10.94	10.79							
Shell thickness (mm)	27	0.33	0.32	0.31	0.33	0.33	0.34	**	***	**	*	NS	*	NS
	35	0.31	0.32	0.33	0.34	0.32	0.34							
	56	0.32	0.33	0.36	0.35	0.33	0.35							
Shell strength (g/cm^2)	27	4426	4518	4532	4844	4657	5058	***	***	***	***	**	***	NS
	35	4096	4092	4234	4485	4366	4691							
	56	4128	3865	4398	4747	4343	4346							
Colour of egg-shell (%)	27	33.91	35.28	41.70	51.58	41.08	49.54	***	***	***	NS	***	***	NS
	35	41.72	40.02	47.56	57.48	47.13	53.72							
	56	41.03	37.33	47.57	52.75	48.59	55.94							

NS = non-significant; *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; K = slow-feathering strains, k = fast-feathering strain a significant ($P \leq 0.01$) interaction between the age and genotype. The egg shape index decreased with the age of hens in all groups with the exception of the slow-feathering hens of the barred variety, in agreement with the conclusions of several other authors (Hamilton et al., 1979). The same authors also stressed the influence of genotype on the egg shape index. However, in our experiment, this indicator was affected by different representation of the feather growth-rate gene within the population, which was demonstrated in a different way depending on the age of birds. The blue variety and the F_1 crossbreeds showed a higher egg shape index in slow-feathering hens at all observed ages. Similarly, a higher egg shape index was found in the barred variety only at the age of 27 weeks.

The eggshell to egg ratio was significantly ($P \leq 0.001$) influenced by age, genotype ($P \leq 0.001$), feather growth-rate gene ($P \leq 0.001$) and by the interaction between the age and the genotype ($P \leq 0.001$). The eggshell to egg ratio decreased at the age of 35 weeks in both strains of Barred Plymouth Rock hens when compared to the values recorded at the age of 27 weeks, then it increased again at the age of 56 weeks. A similar tendency was found in the slow-feathering strain of the Blue Plymouth Rock F_1. In the fast-feathering F_1 hens, the eggshell to egg ratio decreased with the age of hens. These conclusions correspond to a large extent with those of Jelínek (1996), stating that eggshell quality tends to be lower at the beginning of the production period, reaching its best values in the middle of the cycle, and decreasing again towards the end. Comparable conclusions were reported by Hamilton et al. (1979).

The average eggshell thickness (Table 4) was influenced statistically significantly by age ($P \leq 0.001$), genotype ($P \leq 0.001$), and by a significant ($P \leq 0.05$) interaction between the age of hens in the examined groups and the particular genotype. A significant influence ($P \leq 0.01$) was also found for the various representations of the feather growth-rate gene within the population. Eggshell thickness was greatest in the slow-feathering hens of the Barred Plymouth Rock variety, with some thinning at the age of 35 weeks, thickening again at the age of 56 weeks. In the fast-feathering hens, eggshell thickness remained the same and increased at the age of 56 weeks. In the blue variety of Plymouth Rock hens, eggshell thickness increased progressively with production. The findings were different again in both F_1 crossbred strains, where the slow-feathering hens showed a decrease in eggshell thickness at the age of 35 weeks, compared to the thickness at 27 weeks. At the age of 56 weeks, the eggshell thickened again and was comparable to the thickness at 27 weeks. In the fast-feathering strain, eggshell thickness remained the same at the ages of 27 and 35 weeks, followed by thickening at 56 weeks. It should be stated that eggshell thickness was related to the eggshell to egg ratio in most cases. The eggshell results do not correspond very closely to the conclusions drawn by Hamilton (1982) and Doyon et al. (1985). The above-mentioned authors reported identically decreasing eggshell thickness towards the end of the production cycle. The trend was reversed in our experiment, and eggshell thickness increased with the age of hens. Our results tend to correspond with the conclusions of Yannakopoulos and Tserveni-Gousi (1987), reporting thicker shells from older layers.

Eggshell strength, determined by the destruction method, was also significantly ($P \leq 0.001$) influenced by age and genotype ($P \leq 0.001$), with significant differences ($P \leq 0.001$) determined in the interaction between the age of hens and their particular genotype. There was also a significant ($P \leq 0.001$) influence of the feather growth-rate gene and its interaction with the genotype ($P \leq 0.001$). The strongest eggshells were found in eggs produced at the ages of 27 and 35 weeks in the fast-feathering F_1 hens, and at the age of 56 weeks in the fast-feathering hens of the Blue Plymouth Rock variety. In the slow-feathering barred variety, the eggshell strength was greatest at the age of 27 weeks, with lower values at 35 weeks, followed by a mild improvement at 56 weeks of age. In the fast-feathering hens of the Barred Plymouth Rock, eggshell strength gradually decreased with the duration of production. Both strains of Blue Plymouth Rock hens demonstrated a similar situation to the slow-feathering barred variety, while the F_1 crossbreds demonstrated a decrease in eggshell strength with the progression of egg production, comparable to that of the fast-feathering hens of the barred variety. The results indicated considerable variability of this indicator in all observed groups in relation to age. Therefore the experimental results corresponded only partially to the conclusions of some authors who reported a decreasing eggshell strength with the course of the production cycle (Roland, 1984; Jelínek, 1996).

As shown in Table 4, eggshell colour was significantly ($P \leq 0.001$) influenced by age, with a statistically significant ($P \leq 0.001$) influence of the particular genotype. The interaction between the age and the genotype of hens was found insignificant. A significant ($P \leq 0.001$) influence was also demonstrated in the effects of the feather growth-rate gene on the eggshell colour parameter. In the barred variety, the fast-feathering strain had lighter shells at the age of 27 weeks, reversing the trend at the ages of 35 and 56 weeks. Both strains showed the lightest shades of shell colour at the age of 35 weeks. In this case the trend of gradual lightening of eggshell colour with increasing age did not occur. The blue variety of the Plymouth Rock showed significantly ($P \leq 0.001$) lighter shells compared to the barred variety. The fast-feathering strain of the blue variety was found to produce a markedly lighter eggshell, where the values differed by approximately 10% at the ages of 27 and 35 weeks in both strains, and by approximately 5% at the age of 56 weeks. Based on the results for the genotype it was not possible to draw an unambiguous conclusion of increasing eggshell lightness with the layers' age. The slow-feathering strain demonstrated the darkest shades of shells at the age of 27 weeks, with subsequent marked lightening at 35 weeks, remaining nearly the same at 56 weeks of age. The fast-feathering strain of the Blue Plymouth Rock variety produced the darkest eggshells at the age of 27 weeks, the lightest at 35 weeks (with a difference of 5.9%), followed by shell darkening by approximately 5%. Therefore, the experimental results corresponded to the conclusions of Zhang et al. (2005), who identically stated that the eggshell colour lightened with the progression of the production cycle, but only in the case of values determined for the F_1 crossbreds, where the slow-feathering strain showed the marked lightening of shells at 35 weeks, followed by further lightening at the age of 56 weeks. In the fast-feathering F_1 crossbreds, the eggshell tended to lighten uniformly with the progression of the production cycle. The colour of eggs produced by the slow-feathering F_1 crossbreds was nearly identical to that of the slow-feathering strain of the Blue Plymouth Rock used in the sire position. The same conclusion could be applied to the fast-feathering F_1 crossbreds, where the ascertained eggshell colour values corresponded to the values determined for the fast-feathering strain of the Blue Plymouth Rock variety. Therefore the experiment did not support the previous conclusions of the authors who emphasized a stronger influence of the dam on the resultant eggshell colour, as well as a high degree of eggshell colour heritability in brown-egg genotypes (Crawford, 1990; Liu et al., 2001; Hu et al., 2002).

Finally, we state that the age of laying hens, genotype and the feather growth-rate gene incidence within the population significantly affected average egg weight, eggshell quality indicators and eggshell colour. In the case of average egg weight, egg shape index, eggshell to egg ratio, eggshell thickness and strength, an interaction was determined between the age of hens and their particular genotype.

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