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Response of three laying hen genotypes to two dietary calcium levels

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Abstract: The present study aimed to evaluate the effect of two dietary Ca levels (3.00% vs 3.50%) in three hen genotypes (ISA Brown, Bovans Brown and Moravia BSL) on their laying performance and quality of their eggs. In order to evaluate the response to dietary Ca, an experiment was conducted with 300 laying hens. One hundred birds from each genotype were divided into 10 replicates (10 birds/cage/Ca level). To reveal the differences, the following characteristics were measured: hen-day egg production percentage, daily feed intake, egg weight, albumen and yolk weights, Haugh unit, eggshell colour, eggshell weight, eggshell thickness, eggshell strength, egg shape index and eggshell index. A higher Ca level significantly increased the hen-day egg production percentage of Bovans Brown and Moravia BSL, while ISA Brown laying hens had the higher egg production percentage at a lower dietary Ca level (85.97% vs 82.41%). No significant effect of dietary Ca level was observed on any internal egg quality measurements except the Haugh unit. However, the dietary Ca level significantly affected some of external egg quality measurements, namely eggshell colour ($P < 0.001$), eggshell weight ($P = 0.003$) and eggshell strength ($P = 0.012$). The results of the present study show that laying performance and egg quality parameters responded differently according to hen genotype and dietary Ca level. Bovans Brown and Moravia BSL laying hens required higher levels of Ca to keep egg production high, while the eggshell quality measurements were better at the lower dietary Ca level. Thus, it is recommended to adjust the Ca levels in feed to the nutritional requirements of laying hens.

Keywords: dietary Ca; eggshell; genotype; internal quality; performance

In the egg industry, calcium (Ca) is a critical factor ensuring egg production with an acceptable egg quality of laying hens. Ca is the key factor for the structural and metabolic function in bone formation and eggshell quality. Despite the numerous studies of Ca requirements in laying hens, the adequate level of Ca is not clearly estimated. It might be reasoned that several factors affect Ca requirements such as strain, age of birds, etc. An early study was published by Gilbert et al. (1981), who suggested that a 3.00% Ca level was insufficient and 4.50% Ca was recommended to maintain reasonable eggshell quality. A more recent study by Vieira et al. (2011) recommended that 3.41% Ca was the minimum required level in the diet of laying hens.

Castillo et al. (2004) reported that 4.38% and 4.64% were the optimum Ca levels for maximum egg production in the diet of laying hens. Narvaez-Solarte et al. (2006), Safaa et al. (2008) and El-Maksoud (2010) indicated an improvement of egg production with increasing dietary Ca levels. Additionally, an increasing dietary Ca level increased the egg production of laying pigeons (Chang et al. 2019) while the study of Pelicia et al. (2011) concluded a significant linear reduction of

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egg production as dietary Ca levels were increased. Layers fed diets with 4.50% Ca presented lower egg production than those fed 3.00% and 3.75% Ca. However, An et al. (2016) and Tumova et al. (2016) reported no significant effect of dietary Ca levels on egg production. Kermanshahi and Habavi (2006), Pelicia et al. (2011) and Chang et al. (2019) stated that dietary Ca levels did not significantly affect the feed consumption. These different findings might be related to different experimental factors which affected the final outputs.

Several studies have investigated the effect of dietary Ca levels on internal egg quality characteristics. Wu et al. (2007) indicated an increase in albumen and yolk weights with an increasing Ca level, resulting in an increase of egg weight, when the Haugh unit significantly decreased. The authors ascribed these improvements to higher egg weights detected at a higher Ca level. However, An et al. (2016) found no significant effect of Ca levels on the Haugh unit. Chang et al. (2019) reported a significant effect of different dietary Ca levels (0.60, 0.90, 1.20, 1.80, 2.40, and 3.00%) on the egg quality of pigeon eggs. They indicated that albumen percentage, albumen height and Haugh unit were all linearly increased by rising dietary Ca.

Eggshell strength significantly increased when laying hens received more dietary Ca in the study of Jiang et al. (2013). The authors indicated that layers fed a diet with 2.62% Ca had weaker eggshells compared to those on a diet of 3.70% or 4.40% Ca. Moreover, Daniele et al. (2016) reported an increase in the eggshell thicknesses of Japanese quails directly with increasing dietary Ca up to 3.85%. An et al. (2016) reported a linear relationship between dietary Ca level (3.50% vs 4.70%), eggshell strength and eggshell thickness. However, no significant effect of dietary Ca levels (3.20%, 3.70% and 4.20%) on eggshell quality was reported by Swiatkiewicz et al. (2015).

Studies on Ca requirements according to different hen genotypes are insufficient and contradictory. For example, the National Research Council (1994) recommended 3.60% Ca for Brown laying hens, while Hy-Line International (2008) indicated that the Ca requirement of Hy-Line Brown should range from 3.75 to 4.25%. Hence, due to the genetic improvement of laying hens Ca requirements should be changed. In their study on H&N Brown Nick laying hen, Cufadar et al. (2011) did not reveal any significant effect of dietary Ca levels (3.00%, 3.60% and 4.20%) on egg production, egg weight and eggshell quality (namely thickness and strength). Moreover, in Lohmann Brown laying hens, 3.50% and 4.00% Ca affected neither performance nor eggshell quality (Safaa et al. 2008; Tumova et al. 2016).

Since then a considerable amount of research has been conducted on the effect of different dietary Ca levels on the egg quality of laying hens, but only limited information is available on the requirements according to different laying hen genotypes. Therefore, the present experiment was conducted to evaluate the effects of dietary Ca levels on laying performance, internal and external egg quality characteristics of three different laying hen genotypes (ISA Brown, Hisex Brown and Moravia BSL).

MATERIAL AND METHODS

Experimental design, genotype and management. 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.

Three hundred laying hens of ISA Brown, Hisex Brown and Moravia BSL breeds (100 birds per each breed) were kept between 20 and 64 weeks of age and housed in enriched SKN-O 30-60 cages (Kovobel, Domažlice, Czech Republic, 750 cm^2 per hen). All laying hens at the age of 20–40 weeks were fed identical feed mixture N1 (176 g crude protein (CP), 11.6 MJ of metabolizable energy (ME)). From 40 to 64 weeks of age, 150 laying hens (50 of each genotype) received feed mixture N2 (154 g CP, 11.6 MJ ME and 30 g Ca/kg feed) and the other half (150 hens) were fed the same mixture with a higher Ca level (35 g/kg feed). Therefore, during the second half of the laying cycle the experiment was designed to have 10 replicates per each genotype (group of 10 birds/cage/Ca level). Feed and drinking water were available on an *ad libitum* basis. The light schedule consisted of 16 h light and 8 h darkness (Souza et al. 2019).

Laying performance and internal egg quality measurements. Hen-day egg production was calculated using the data of daily recorded egg production as the number of eggs produced during the period/number of hens in the period × 100

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(Sekeroglu et al. 2014). Totally 2421 eggs were collected and weighed individually with an electronic balance (± 0.01 g).

Internal egg qualities were evaluated as follows: yolk weight was evaluated using a sensitive balance after separation from albumen. Albumen weight was calculated by subtracting yolk weight and eggshell weight from egg weight. The yolk albumen ratio was calculated as (yolk weight/albumen weight) × 100. The albumen height and Haugh unit (Haugh 1937) were evaluated using a QCH albumen height gauge (TSS, UK).

External egg quality measurements. Eggs for eggshell quality assessments were evaluated for the eggshell colour which was measured with a QCR shell colour reflectometer (TSS). The reflectometer works by taking a percentage reading between black and white with the former expressed as 0% and the latter pure white 100%. The egg shape index was calculated after measuring the length and width of each individual egg (width/length × 100). Eggshell strength was determined by the shell-breaking method using a QC-SPA system (TSS). After measuring eggshell strength, the eggs were broken and the internal egg components were kept for further analysis. Eggshell thickness was measured with a QCT shell thickness micrometer (TSS) at the equatorial region after removal of shell membranes (Ketta and Tumova 2018a). Eggshell weight was determined after complete drying. The eggshell index was calculated according to the following equation (Ahmed et al. 2005): Eggshell index = (shell weight/shell surface) × 100, where the surface area of each egg was calculated using the formula (egg surface = 4.67 (egg weight)$^{2/3}$) as suggested by Thomson et al. (1985).

Statistical analyses. The results of the experiment were evaluated by two-way ANOVA using the GLM procedure of SAS (Version 9.4, 2013). The model included the effects of genotype and dietary Ca level, as well as the interactions among them. $P < 0.05$ was considered statistically significant. The relationship between laying performance, external and internal egg quality measurements was evaluated by estimating Pearson's correlation coefficient.

RESULTS AND DISCUSSION

In terms of the hen laying performance, the three laying hen genotypes used in the present study responded differently to dietary Ca levels (Table 1). Increasing the dietary Ca from 3.00% to 3.50% significantly increased the hen-day egg production percentage of Bovans Brown and Moravia BSL. Similarly, Safaa et al. (2008) and El-Maksoud (2010) found an improvement of egg production with increasing dietary Ca levels. The lower egg production percentage of Bovans Brown at a lower dietary Ca level (62.38%) could be due to the consumption of eggs by birds to compensate the lack

Table 1. Effects of dietary Ca levels and genotype and their interactions on hen performance measurements and egg weight

Factors		Measurements		
		hen-day egg production (%)	daily feed intake (g)	egg weight (g)
ISA Brown	3.00% Ca	85.97^{a}	145.13	61.76
	3.50% Ca	82.41^{ab}	148.16	61.18
Bovans Brown	3.00% Ca	62.38^{c}	168.37	63.61
	3.50% Ca	80.33^{ab}	182.05	62.83
Moravia BSL	3.00% Ca	72.67^{bc}	204.82	62.07
	3.50% Ca	75.94^{b}	167.82	62.83
RMSE		19.770	67.080	6.090
Genotype		0.005	0.002	0.001
Ca level		0.034	0.472	0.441
Genotype × Ca level		0.006	0.069	0.063

RMSE = root mean square error

$^{a–c}$means in the same column with different superscripts differ at $P < 0.05$

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of Ca in feed. This theory might also explain the higher eggshell quality measurements of Bovans Brown obtained in the present study. However, ISA Brown laying hens had higher egg production at the lower dietary Ca level compared to the higher one. Our observation is in agreement with Pelicia et al. (2011), who reported that ISA Brown fed 3.00% Ca had higher egg production (91.3%) compared to 3.75% Ca (90.6%) and 4.50% Ca (87.6%). It might be assumed that ISA Brown hens had the ability to utilise more Ca from the feed that had lower Ca content. The individual effect of hen genotype was significant for all performance parameters. ISA Brown had the highest hen-day egg production ($P = 0.005$) and the lowest daily feed intake ($P = 0.002$), while Bovans Brown produced the heaviest eggs ($P < 0.001$). Moravia BSL fed 3.00% Ca had slightly higher daily feed intake (204.82 g) than Bovans Brown (168.37 g) and ISA Brown (145.13 g). It might be explained that Moravia BSL compensated for lower dietary Ca by increasing the feed intake. The individual effect of dietary Ca level on feed intake and egg weight was not significant in the present study. Our results are in agreement with Pelicia et al. (2011) and An et al. (2016), who reported no significant effect of dietary Ca level on either feed intake or egg weight.

The effect of dietary Ca level on the internal egg quality of the three laying hen genotypes is presented in Table 2. No significant effect of dietary Ca level on all internal egg quality measurements except the Haugh unit was observed. Contrary observations were reported by Wu et al. (2007), Skrivan et al. (2018) and Chang et al. (2019), who found an improvement in internal egg quality measurements when a dietary Ca level was increased. These different observations might be attributed to the use of different genotypes or to different environmental conditions in which the hens were kept. Albumen weight was not affected by the interactions of genotype and Ca level. However, the interactions between evaluated factors were detected for yolk weight ($P < 0.001$), yolk/albumen ratio ($P < 0.001$) and Haugh unit ($P < 0.001$) in the present study. Moravia BSL fed 3.50% Ca laid eggs with heavier yolk while the yolk weight of ISA Brown eggs at 3.50% Ca was the lowest. The results indicated different responses of laying hen genotypes according to the Ca level in feed. Eggs from ISA Brown and Bovans Brown showed higher yolk weight and yolk/albumen ratio at 3.00% Ca. However, at 3.50% Ca level, Moravia BSL had heavier yolk and higher yolk/albumen ratio.

Regarding the eggshell quality measurements, the three laying hen genotypes responded differently according to the Ca levels in feed (Table 3). The dietary Ca level significantly affected eggshell colour ($P < 0.001$), eggshell weight ($P = 0.003$) and eggshell strength ($P = 0.012$). However, the Ca content in feed did not affect the rest of the eggshell quality measurements. Our results are in accordance with Swiatkiewicz et al. (2015), who indicated that different Ca levels (3.20%, 3.70% and 4.20%) did not affect the eggshell quality measurements. However, an increase in eggshell

Table 2. Effects of genotype and dietary Ca levels and their interactions on egg internal quality measurements

Factors		Measurements			
		albumen weight (g)	yolk weight (g)	yolk/albumen ratio (%)	Haugh unit score
ISA Brown	3.00% Ca	39.32	15.26^{bc}	38.99^{b}	82.68^{c}
	3.50% Ca	39.01	14.92^{c}	38.53^{c}	84.35^{bc}
Bovans Brown	3.00% Ca	39.54	16.12^{ab}	41.11^{ab}	86.07^{b}
	3.50% Ca	39.89	15.35^{bc}	38.67^{bc}	86.86^{ab}
Moravia BSL	3.00% Ca	39.51	15.60^{b}	39.52^{ab}	91.46^{a}
	3.50% Ca	39.30	16.40^{a}	41.81^{a}	85.52^{b}
RMSE		4.272	2.204	5.595	11.114
Genotype		0.026	0.001	0.001	0.001
Ca level		0.770	0.271	0.396	0.015
Genotype × Ca level		0.250	0.001	0.001	0.001

RMSE = root mean square error

$^{a–c}$means in the same column with different superscripts differ at $P < 0.05$

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Table 3. Effects of genotype and dietary Ca levels and their interactions on eggshell quality measurements

Factors		Measurements					
		shell colour (%)	shell weight (g)	shell strength (g/cm^2)	shell thickness (mm)	egg shape index (%)	shell index (g/100 cm^2)
ISA Brown	3.00% Ca	31.9^b	6.0^b	4542^b	0.365	77.4^b	9.8^b
	3.50% Ca	31.5^b	6.1^b	4718^{ab}	0.357	77.0^c	10.0^{ab}
Bovans Brown	3.00% Ca	28.7^c	6.7^a	4993^a	0.382	77.4^b	10.7^a
	3.50% Ca	30.8^{bc}	6.3^{ab}	4679^{ab}	0.365	77.6^a	10.3^{ab}
Moravia BSL	3.00% Ca	43.5^{ab}	5.7^c	4457^{bc}	0.328	77.0^c	9.5^c
	3.50% Ca	44.4^a	5.8^c	4322^c	0.330	77.1^c	9.6^c
RMSE		5.020	0.690	842	0.186	2.800	0.730
Genotype		0.001	0.001	0.001	0.001	0.003	0.001
Ca level		0.001	0.003	0.012	0.317	0.427	0.273
Genotype × Ca level		0.001	0.001	0.001	0.678	0.031	0.001

RMSE = root mean square error

$^{a-c}$means in the same column with different superscripts differ at $P < 0.05$

thickness and strength, when dietary Ca increased, was reported by An et al. (2016) and Daniele et al. (2016).

Significant interactions between genotype and dietary Ca level were detected for the eggshell colour when Bovans Brown fed 3.00% dietary Ca had the darkest shells (28.7%). Yang et al. (2009) reported a high correlation between dark eggshell colour and eggshell quality, which might also explain the higher eggshell quality measurements for Bovans Brown obtained in the present study. Eggshell weight, strength and eggshell index of ISA Brown eggs were significantly increased by an increasing dietary Ca level while different reactions were observed for Bovans Brown and Moravia BSL. The previously mentioned eggshell measurements were significantly higher in hens fed the low level of Ca (3.00%) compared with the high level (3.50%). Eggshell weight, strength and shell index values of Bovans Brown fed 3.00% Ca were the highest (6.70 g, 4993 g/cm^2, 10.70 g/cm^3, respectively), while the eggshells of Moravia BSL had the lowest values (5.70 g, 4322 g/cm^2, 9.50 g/cm^3, respectively).

Pearson's correlation coefficients of some selected laying performance, external and internal egg quality measurements (Table 4) indicated no significant correlation between laying performance and external and internal egg measurements. However, the results indicated negative correlations between hen-day egg production and eggshell quality measurements, namely eggshell weight, shell thickness and shell strength. Similarly, Dunn et al. (2005) reported a negative correla-

Table 4. Pearson's correlation coefficients between selected laying performance, external and internal egg quality measurements

	Hen-day egg production	Feed intake	Egg weight	Eggshell weight	Shell strength	Shell thickness	Albumen weight
Feed intake	–0.06						
Egg weight	0.02	–0.08					
Eggshell weight	–0.09	–0.06	0.63***				
Shell strength	–0.07	0.09	–0.05*	0.37***			
Shell thickness	–0.05	–0.04	0.03	0.14***	0.10***		
Albumen weight	0.05	–0.02	0.92***	0.52***	–0.05*	0.02	
Yolk weight	–0.02	–0.16	0.73***	0.41***	–0.015***	0.01	0.43***

significant at *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$)

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tion between egg production and eggshell quality. Highly positive correlations between egg weight and eggshell weight (r^2 = 0.63), albumen weight (r^2 = 0.92) and yolk weight (r^2 = 0.73) were found in the present study. All these genetic relations can be used to improve egg quality through selection according to the weight of eggs. Eggshell strength and thickness were significantly correlated (r^2 = 0.10). These findings are in agreement with Kibala et al. (2015) and Ketta and Tumova (2018b), who reported positive genetic correlations between eggshell strength and its thickness making them indicators for each other.

CONCLUSION

The results of the present study showed that laying hen genotypes differ in their ability to utilize Ca from feed. To maintain higher egg production, Bovans Brown and Moravia BSL laying hens required higher levels of Ca, while the eggshell quality measurements were better at the lower dietary Ca level. In ISA Brown, higher egg production and lower shell measurements were obtained at the lower dietary Ca level. Therefore, it is highly recommended to estimate the level of Ca in feed according to the requirements of laying hens.

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