doi: 10.17221/3/2016-CJAS

Effect of dietary magnesium, calcium, phosphorus, and limestone grain size on productive performance and eggshell quality of hens

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ABSTRACT: Two experiments conducted on laying hens, aged 50 and 52 weeks, were carried out to evaluate the effect of dietary Mg and its relationship to the Ca : NPP (non-phytate phosphorus) ratio using a various grain-size of limestone. The Ca : NPP ratio in each experiment was 12.8 and 18, respectively. Two Mg levels were evalued in the first (1.56 and 4.0 g/kg) and the second experiment (1.52 and 4.0 g/kg). A fine-grained limestone (< 0.5 mm; FL) or a coarse-grained limestone (0.8–2 mm; CL) was used in the first experiment. In the second experiment, a mixture of both the aforementioned limestone forms (FCL) was used as the third alternative. The main parameters estimated in this study were egg production and egg shell breaking strength. In the first experiment, CL significantly increased hen-day egg production ($P = 0.043$) and Mg (in dietary concentration up to 4 g/kg) increased egg weight ($P < 0.001$). The addition of Mg to the mixed feed, together with CL, decreased yolk percentage ($P = 0.008$), increased egg shell percentage ($P = 0.044$), increased egg shell thickness ($P = 0.014$), and egg shell breaking strength ($P = 0.003$). Higher dietary Mg, together with a wider Ca : NPP ratio in the second experiment, increased egg production and egg weight ($P < 0.001$), but it did not influence egg shell breaking strength. CL increased egg shell breaking strength compared to the addition of FL, as well as FCL ($P < 0.05$), regardless of the Mg levels. Lower level of Mg with FL decreased ash content of shells ($P = 0.004$).

Keywords: magnesium oxide; limestone granularity; mixed feed; Ca : NPP ratio; egg shell strength; layers

INTRODUCTION

The productivity of modern layer strains has improved significantly as a result of genetic and nutritional improvements. However, the quality of eggshells, especially in aged hens, is low. Most research has been focused on the concentration of Ca in diets because Ca is the major mineral in the eggshell, and its concentration directly affects eggshell quality (Scott et al. 1970; Kim et al. 2013). The National Research Council (1994) estimated the requirement for Ca, non-phytate phosphorus (NPP), and Mg at 110 g of feed per brown-egg layer daily as 3.6 g, 0.275 g, and 0.055 g, respectively. Dietary Mg concentration has been associated with laying performance and eggshell quality (Stafford and Edwards 1973; Ding and Shen 1992; Venglovska et al. 2014). Previous experiments indicated that the eggshell strength improved in laying hens when the Mg concentration in their diet was increased above the Mg nutrition requirement (Atteh and Leeson 1983a, b; Hess and Britton 1997; Kim et al. 2013).

Mg metabolism is closely associated with Ca and P (McDonald et al. 2011). The antagonistic relationship also seems to exist between Ca and Mg in relation to skeletal integrity and eggshell quality in laying hens (Shastak and Rodehutscord 2015). Toba et al. (1999) reported that when

Supported by the Ministry of Agriculture of the Czech Republic (Project No. QJ1310002 and Project No. MZeRO0714).

doi: 10.17221/3/2016-CJAS

growing male rats were fed three times more Mg compared to the control group, an increase in bone-breaking energy was observed. Toba et al. (2000) also reported that Mg supplementation in ovariectomized rats promoted bone formation by activating osteoclasts. High Mg supplementation may cause laxative problems. Stillmak and Sunde (1971) stated that an excess of Mg (1% in the diet) reduced dry matter of faeces, accelerated the passage of digesta, and caused a deficit of Ca. Excess of Mg in hens can also reduce the activity of parathyroid hormone, which reduces blood Ca and subsequently also egg production and shell quality (Hess and Britton 1997).

The available phosphorus (AP) requirement from previously published scientific studies has varied from 0.13 to 0.30% (Miles et al. 1983; Summers 1995; Scott et al. 1999; Sohail and Roland 2002, a.o.). Skrivan et al. (2010) showed that 0.27% AP in wheat-based and 0.30% AP in maize-based diets without added phytase were adequate for hens with an intake of 115 g of feed that contained 3.5% Ca, without negative effects on the performance or egg quality. Results of Snow et al. (2004) indicated that first-cycle hens required approximately 0.18% AP or 198 mg AP per hen/day. In the study by Kozlowski and Jeroch (2011), two very different levels of NPP (2.5 and 1.3 g/kg) were compared; the authors concluded that the decrease in the NPP content resulted in a significant reduction in the laying rate and egg mass by approximately 3.9% and 5.4%, respectively, and significantly worsened the feed conversion efficiency. A high dietary concentration of NPP is related to the decrease of productive performance of layers. Englmaierova et al. (2012) found that the hens fed a diet with only 1.3 g/kg NPP achieved a higher performance than the hens with 4.0 g/kg NPP in the diet.

Currently, research of nutritional effects on the quality of eggshells has been preferably focused on Ca. The grain size of limestone is also significant. Skrivan et al. (2010) described that 1–2 mm grains of limestone increase performance of hens and thickness of the eggshell. Similarly, a study by Wang et al. (2014) with the same limestone grain size found positive effects on the strength of the eggshell in ducks. Therefore, it can be hypothesized that the effects of the dietary addition of Mg on the quality of eggshell may be related to the grain-size distribution of limestone and the mutual relationship between dietary concentration of Ca and NPP. However, this hypothesis has not been tested.

The objective of this experiment, therefore, was to determine the effect of increasing levels of Mg in diets with different grain sizes of limestone, as well as the effect of different Ca : NPP ratios on productive performance and eggshell quality in hens.

MATERIAL AND METHODS

Experiment 1. Two hundred and forty 50-week-old Lohmann Brown hens were randomly distributed into 4 dietary treatments with 6 replicate cages at 10 hens per cage. The hens were housed in the same air-conditioned facility in three-floor enriched cages. The cages were equipped with a nest box, perch (150 cm), dust bath, and equipment for the abrasion of claws, conforming to the Council Directive 1999/74/EC. The cage provided 7560 cm^2 of floor area without the nest, 120 cm of feeder, and 3 nipple water dispensers. The room temperature was maintained at 20–22°C. A 16-h photoperiod was applied, and the light intensity was approximately 10 lx in the central storey.

A completely randomized experimental design with a 2 × 2 factorial arrangement of treatments was employed: 2 Mg levels (1.56 and 4.0 g/kg) and 2 types of limestone grain size (fine-grained limestone (FL; < 0.5 mm) and coarse-grained limestone (CL; 0.8–2 mm)) in mixed feed. The concentration of Mg in the diet was increased by the addition of MgO. The ingredients and nutrient composition of the basal diet are listed in Table 1. The Ca : NPP ratio was 12.8. Feed and fresh water were supplied *ad libitum*. The experiment lasted for 12 weeks.

The number of eggs and hens and their health status were monitored daily. The hen-day egg production and feed intake were calculated weekly on a per-cage basis. Egg weights were determined once per week.

The feed was analyzed as described previously (Skrivan et al. 2015). The total P in the diets was assayed using a vanadate–molybdate reagent according to the AOAC method 965.17 (AOAC 2005). The Ca and Mg contents in the diets were determined by atomic absorption spectrometry performed using a Solaar M6 instrument (TJA Solutions, Cambridge, UK). The phytate P contents of the diets were determined by a capillary isotachophoretic method (Duskova et al. 2001).

doi: 10.17221/3/2016-CJAS

Table 1. Ingredients and determined chemical composition of hen basal diets

Ingredients (g/kg)	Experiment	
	1	2
Maize	360	369.4
Wheat	280	280
Soybean meal	230	230
Rapeseed oil	20	20
Limestone[1]	91	87
Dicalcium phosphate	9	3.6
NaCl	2.5	2.5
Vitamin/mineral premix[2]	5	5
L-Lysine hydrochloride	1	1
DL-Methionine	1.5	1.5
Composition		
Dry matter	895	890
Crude protein	165	170
Calcium (Ca)	37	36
Total phosphorus	5.1	4.6
Non-phytate phosphorus (NPP)	2.9	2.0
Magnesium[3]	1.56	1.52
Ca : NPP	12.8	18
Calculated ME (MJ/kg)	11.36	11.40
Calculated LYS	8.8	8.8
Calculated TSAA	7.5	7.5

ME = metabolizable energy, LYS = lysine, TSAA = total sufur amino acids

[1]experimental diet contained 100% fine-grained limestone (FL) or 100% coarse-grained limestone (CL) in Experiment 1, and 100% fine-grained limestone or 100% coarse-grained limestone or 35% fine-grained limestone and 65% coarse-grained limestone (FCL) in Experiment 2

[2]Vitamin/mineral premix provided per kg of diet: retinylacetate 3.0 mg, vitamin D_3 3000 IU, vitamin E 30 mg, niacin 25 mg, Ca pantothenate 8 mg, thiamine 2.0 mg, riboflavin 5 mg, pyridoxine 4 mg, folic acid 0.5 mg, biotin 0.075 mg, cobalamin 0.01 mg, choline chloride 250 mg, menadione 2.0 mg, betaine 100 mg, butylated hydroxytoluene 7.5 mg, ethoxyquin 5.6 mg, butylhydroxyanisole 1 mg, DL-methionine 0.7 g, Mn 70 mg, Zn 50 mg, Fe 40 mg, Cu 6 mg, I 1 mg, Co 0.3 mg, Se 0.2 mg

[3]concentration of Mg in diet was increased to 4 g/kg by addition of MgO

For the determination of the physical parameters of the eggs, the eggs were collected three times during the experiment (Tumova et al. 2014; Englmaierova et al. 2015). All daily egg production from one day in each collection period was analyzed. Haugh units (HU) were calculated according to Haugh (1937). Shell breaking strength was determined on the vertical axis using an Instron 3360 apparatus (Instron, Norwood, USA). Shell thickness (i.e. the average of the 3 values from the sharp and blunt ends and the equator) was measured using a micrometre, after removing the shell membranes. The shells with membranes were washed, dried for 2 h at 60°C, and weighed. The albumen, yolk, and shell percentages were determined using the individual weight of each egg and the weights of its components.

Analyses of the P, Ca, and Mg contents of the eggshells were conducted twice during the experiment ($n = 24$). Dry samples of the eggshells were ashed at 550°C. The P, Ca, and Mg contents of the dried eggshells were determined in a manner similar to that described previously for the analysis of these elements in the hens' diets.

Experiment 2. A total of one hundred and twenty-six 52-week-old Lohmann Brown hens were housed individually in three-floor cages (21 hens per treatment) in the same air-conditioned facility. The cages provided 2000 cm^2 of floor area and had a 40 cm feeder and 2 nipple water dispensers. The room temperature was maintained at 20–22°C, and the daily photoperiod was 16 h light/8 h darkness with a light intensity of approximately 10 lx in the central storey. The hens were randomly assigned to 6 dietary treatments. The study was a 2 × 3 full factorial design with 2 levels of Mg (1.52 and 4.0 g/kg; NPP), 3 types of limestone grain size (FL (< 0.5 mm), CL (0.8–2 mm), and a mix of FL and CL (FCL; 35 : 65)) in mixed feed. The ingredients and nutrient composition of the basal diet are listed in Table 1. The Ca : NPP ratio was 18. The feed and fresh water were supplied *ad libitum*. The experiment was conducted for 12 weeks.

Determination of the performance characteristics of the hens, the physical parameters of the eggs, and the P, Ca, and Mg contents of the diets and eggshells were the same as described previously in Experiment 1.

Statistical analysis. The data from both experiments were analyzed using two-way analysis of variance (ANOVA) with the General Linear Models (GLM) procedure of the SAS software (Statistical Analysis System, Version 9.3, 2003). The main effects considered were the concentration of magnesium (Mg), the grain size of limestone (L), and the interaction between these two factors

doi: 10.17221/3/2016-CJAS

(Mg × L). The significance differences between groups were determined by Scheffé's test. All of the differences were considered to be significant at $P < 0.05$. The results in the tables are presented as the mean and standard error of the mean (SEM).

RESULTS

In the diet of Experiment 1, there was a narrow ratio of Ca : NPP (12.8). As is shown in Table 2, an effect of L was found: CL increased egg production ($P = 0.043$) contrary to FL. The higher level of Mg increased egg weight ($P < 0.001$) and decreased feed conversion ratio ($P = 0.049$). Table 3 summarizes the results of physical characteristics of egg quality. The Mg addition together with CL increased shell thickness ($P = 0.014$), shell percentage ($P = 0.044$), and shell strength ($P = 0.003$) and decreased yolk percentage ($P = 0.008$). In addition, higher level of Mg in diet increased albumen percentage ($P = 0.007$). Fine limestone increased Ca content in ash of the shell ($P < 0.001$) contrary to CL (Table 4). The P and Mg in the shell were not influenced by experimental treatments.

In Experiment 2, there was a wider ratio of Ca : NPP (18). In performance characteristics showed in Table 5, there was a significant interaction of Mg and L for egg weight ($P < 0.001$). The highest values were recorded in the case of Mg at concentration of 1.52 g/kg with FCL (66.3 g) and Mg

Table 2. Performance parameters of laying hens – Experiment 1

	1.56 (g Mg/kg diet)		4.0 (g Mg/kg diet)		SEM	Probability		
	FL	CL	FL	CL		Mg	L	Mg × L
Hen-day egg production (%)	82.0	85.0	83.2	85.1	0.57	ns	0.043	ns
Egg weight (g)	65.6	65.8	66.9	67.3	0.12	< 0.001	ns	ns
Feed intake (g/day/hen)	116.3	115.2	114.8	112.4	0.60	ns	ns	ns
Feed conversion ratio (g feed/g eggs)	2.11	2.04	2.03	1.95	0.021	0.049	ns	ns

L = limestone, FL = fine-grained limestone, CL = coarse-grained limestone, ns = not significant

Table 3. Physical characteristics of egg quality – Experiment 1

	1.56 (g Mg/kg diet)		4.0 (g Mg/kg diet)		SEM	Probability		
	FL	CL	FL	CL		Mg	L	Mg × L
Haugh units	81.2	83.1	82.2	83.3	0.58	ns	ns	ns
Albumen percentage (%)	64.4	64.5	64.8	65.8	0.13	0.007	ns	ns
Yolk percentage (%)	25.4^a	25.5^a	25.0^a	23.7^b	0.12	< 0.001	ns	0.008
Shell thickness (μm)	371^b	370^b	376^b	393^a	1.7	< 0.001	ns	0.014
Shell breaking strength (g/cm^2)	4204^b	4203^b	4165^b	4733^a	44.0	ns	ns	0.003
Shell percentage (%)	10.2^b	10.1^b	10.2^b	10.5^a	0.05	ns	ns	0.044

L = limestone, FL = fine-grained limestone, CL = coarse-grained limestone, ns = not significant

$^{a–b}$means within the same row with different superscripts differ significantly

Table 4. Mineral composition of eggshells – Experiment 1

	1.56 (g Mg/kg diet)		4.0 (g Mg/kg diet)		SEM	Probability		
	FL	CL	FL	CL		Mg	L	Mg × L
Ash content of shell (g/kg DM)	951	951	952	948	0.05	ns	ns	ns
Shell Ca content (g/kg DM)	391	378	390	379	1.8	ns	< 0.001	ns
Shell P content (g/kg DM)	1.26	1.28	1.24	1.30	0.015	ns	ns	ns
Shell Mg content (g/kg DM)	4.22	4.26	4.49	4.75	0.049	ns	ns	ns

L = limestone, FL = fine-grained limestone, CL = coarse-grained limestone, DM = dry matter, ns = not significant

doi: 10.17221/3/2016-CJAS

Table 5. Performance characteristics of laying hens – Experiment 2

	1.52 (g Mg/kg diet)			4.0 (g Mg/kg diet)			SEM	Probability		
	FL	CL	FCL	FL	CL	FCL		Mg	L	Mg × L
Hen-day egg production (%)	89.7	90.4	88.4	92.7	93.6	93.7	0.44	< 0.001	ns	ns
Egg weight (g)	63.8[c]	64.8[b]	66.3[a]	66.8[a]	66.4[a]	65.2[b]	0.13	< 0.001	ns	< 0.001
Feed intake (g/day/hen)	116.9	112.8	111.8	118.7	119.8	113.1	0.58	0.002	< 0.001	ns
Feed conversion ratio (g feed/g eggs)	2.05	1.94	1.92	1.92	1.93	1.85	0.012	0.005	0.002	ns

L = limestone, FL = fine-grained limestone, CL = coarse-grained limestone, ns = not significant
[a–b]means within the same row with different superscripts differ significantly

Table 6. Physical characteristics of egg quality – Experiment 2

	1.52 (g Mg/kg diet)			4.0 (g Mg/kg diet)			SEM	Probability		
	FL	CL	FCL	FL	CL	FCL		Mg	L	Mg × L
Haugh units	83.1	82.1	82.1	79.7	81.7	79.8	0.48	0.033	ns	ns
Albumen percentage (%)	64.3	63.8	64.7	64.0	64.3	64.5	0.13	ns	ns	ns
Yolk percentage (%)	26.3	26.6	25.7	26.4	26.2	25.8	0.11	ns	0.049	ns
Shell thickness (μm)	332	340	342	342	339	341	1.6	ns	ns	ns
Shell breaking strength (g/cm^2)	3500	3926	3613	3484	3702	3634	40.1	ns	0.004	ns
Shell percentage (%)	9.5	9.6	9.6	9.6	9.5	9.7	0.04	ns	ns	ns

L = limestone, FL = fine-grained limestone, CL = coarse-grained limestone, ns = not significant

Table 7. Mineral composition of eggshells – Experiment 2

	1.52 (g Mg/kg diet)			4.0 (g Mg/kg diet)			SEM	Probability		
	FL	CL	FCL	FL	CL	FCL		Mg	L	Mg × L
Ash content of shell (g/kg DM)	942[b]	949[a]	954[a]	951[a]	952[a]	948[a]	0.9	ns	0.038	0.004
Shell Ca content (g/kg DM)	373	373	375	372	373	362	1.4	ns	ns	ns
Shell P content (g/kg DM)	1.22	1.27	1.30	1.08	1.23	1.13	0.021	0.006	ns	ns
Shell Mg content (g/kg DM)	3.82	3.56	3.75	3.93	3.96	3.80	0.050	0.036	ns	ns

L = limestone, FL = fine-grained limestone, CL = coarse-grained limestone, DM = dry matter, ns = not significant
[a–b]means within the same row with different superscripts differ significantly

at concentration of 4 g/kg with FL (66.8 g) or CL (66.4 g). The addition of Mg increased egg production ($P < 0.001$) and feed intake ($P = 0.002$) and decreased feed conversion ratio ($P = 0.005$). A lower feed intake ($P < 0.001$) and feed conversion ratio ($P = 0.002$) was ascertained in the diet with FCL.

As is evident from Table 6, the shell strength increased due to CL, but it did not increase with FL or the combination of these two types of limestone ($P < 0.004$). The FCL in diet decreased yolk percentage ($P = 0.049$). Higher level of Mg had a negative effect on Haugh units ($P = 0.033$). Significant interaction of Mg concentration and type of grain size of L ($P = 0.004$) was found in ash content of shell (Table 7). Lower level of Mg with FL decreased ash content compared to other evaluated groups. The added Mg increased the Mg content ($P = 0.036$) and decreased P content in the shell ($P = 0.006$) but did not affect the concentration of Ca in the shell.

DISCUSSION

The present study describes the effects of dietary Mg, which was added into feed mixtures together

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with FL, CL (0.8–2.0 mm), or with both types of L. Both narrow and wide Ca : NPP ratios were used. The Mg contents in the basal feed mixture in Experiment 1 and 2 were 1.56 and 1.52 g/kg, respectively. This was thrice more than the nutritional requirement, which is 0.5 g Mg/kg (National Research Council 1994). Magnesium at higher dosing up to 4 g/kg together with CL and at narrow Ca : NPP ratio significantly increased the shell percentage, shell thickness, and shell strength. Previous studies showed that Mg supplementation above 5 g/kg, at 4.7 g/kg, or at 3 g/kg increased the eggshell quality in laying hens (Mehring and Johnson 1965; West et al. 1980; Kim et al. 2013). In Experiment 1, the Mg supplementation increased Mg concentration in the eggshell and decreased Ca concentration. This is in agreement with data by Atteh and Leeson (1983b). Also in Experiment 2, at the wide dietary Ca : NPP ratio, the Mg concentration in the eggshell was higher when Mg was added into the diet. The eggshell Ca concentration insignificantly decreased when Mg was added into the diet with FCL. Therefore, our results are in agreement with data from Atteh and Leeson (1983b) regarding Mg and in partial agreement regarding Ca since a wide Ca : NPP ratio and FCL may influence resorption and metabolism of Ca and consequently also Ca deposition in the eggshell. Both high eggshell strength and eggshell thickness due to an increased dietary Mg concentration of 4 g/kg in the experiment with a narrow Ca : NPP ratio are consistent with data from Kim et al. (2013). These authors did not observe effects of the dietary Mg concentration up to 3 g/kg on the laying intensity, egg mass, and feed conversion. The difference between their results and ours concerning the laying intensity could be related to the Ca : NPP ratio. When the Ca : NPP ratio was narrow, such as in Experiment 1 and in the study by Kim et al. (2013), the dietary Mg did not influence the laying intensity, but it increased the eggshell strength. A wide Ca : NPP ratio of 19.5 : 1 and 24 : 1 without Mg supplementation significantly reduced laying intensity in the experiment of Englmaierova et al. (2014). A similar relationship was seen in Experiment 2. We observed that Mg, which is the Ca antagonist, improved the Ca, Mg, and P ratios and reduced the excess Ca in relationship with P. The eggshell strength was improved due to the CL influence in comparison with FL and FCL. A high Ca : P ratio in broiler chickens reduced the digestibility and utilization of Ca and P (Olukosi and Fru-Nji 2014). A high Ca concentration relative to P increased the formation of Ca-phytate complexes (Wise 1983). The Mg supplementation did not decrease the feed intake in our experiments. This possibility has been proposed by Kim et al. (2013) in the case of MgO supplementation, but it has not been proposed when $MgCl_2$, $MgCO_3$, and $MgSO_4$ were supplemented. Additionally, the amount of Mg should be taken into account as the authors indicated that the MgO supplement up to 3 g Mg/kg did not decrease the feed intake and hen performance. This is consistent with our results when the Mg supplementation was distinctly higher.

The CL containing 0.8–2 mm particles showed positive effects on the performance of hens, eggshell thickness, and eggshell strength (see Skrivan et al. 2010). Wang et al. (2014) successfully used L of the same structure in the diet of laying ducks. They achieved significantly better performance (greater egg mass, better feed conversion, higher eggshell strength, albumen height, Haugh units, and P and Mg concentration in the shell). The positive effect of CL could be caused by the fact that larger particle size of L with lower *in vitro* solubility was retained in the gizzard for a longer time which may increase Ca retention and improve the shell quality. The dietary combination of CL, FL and their mixture together with increased Mg in Experiment 2 ameliorated laying performance and egg mass. The eggshell strength was not influenced at a wide Ca : NPP ratio. However, there may be a shortage of Ca at a high laying intensity and high egg mass. The antagonism of Ca and Mg cannot be underestimated (Shastak and Rodehutscord 2015). When the dietary Mg concentration was not increased, CL increased the eggshell strength, even at a wide Ca : NPP ratio. Fine limestone and the CL and FCL supplement in groups with higher Mg concentration reduced P in the eggshell, whereas dietary CL together with Mg insignificantly increased Mg in the eggshell at the wide Ca : NPP ratio.

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Received: 2016–01–21
Accepted after corrections: 2016–06–28

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