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## Effects on egg production and quality of supplementing drinking water with calcium and magnesium

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### Abstract

This study was conducted to appraise the effects on egg quality and production performance of laying hens when drinking water was supplemented with calcium (Ca) and magnesium (Mg). A total of 384 (64-week-old) Hy-line Brown laying hens were assigned at random to four treatments, which consisted of CON: unsupplemented drinking water; T1: drinking water + 2 mg/L Ca + 250 mg/L Mg; T2: drinking water + 4 mg/L Ca + 510 mg/L Mg /10 L; and T3: drinking water + 5 mg/L Ca and 760 mg/L Mg. The experiment lasted six weeks. Water intake increased linearly in week 1 with the rising levels of Ca and Mg in the drinking water. Increasing the Ca and Mg levels improved eggshell strength (week 2 ( $P=0.01$ ), week 5 ( $P=0.01$ ), and week 6 ( $P=0.03$ ), and eggshell thickness (week 6) ( $P=0.02$ ) and reduced the rate at which eggs were broken (week 4) ( $P=0.01$ ). The supplemental Ca and Mg did not affect egg production, egg weight, Haugh unit, albumen height, eggshell colour, and yolk colour compared with CON. Nor did they influence the Haugh unit and albumen height after storing for 1, 5, 10 and 15 days. In conclusion, adding Ca and Mg to the drinking water increased the thickness and strength of the eggshells.

**Keywords:** eggshell, laying hens, minerals, poultry, water quality,

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### Introduction

Water is important in many body functions in laying hens, including digestion, absorption, maintenance of ionic balance, excretion of waste materials, provision of media to transport nutrients, heat regulation and metabolism of nutrients (Schlink *et al.*, 2010). Poor water quality can affect hen performance negatively, even when a well-balanced diet is supplied (Chung *et al.*, 2020). High levels of dissolved minerals in water may have negative effects on poultry performance and health (Leeson & Summers, 2008). For example, Ca is the major mineral in eggshells and bones (Whitehead, 2004), but excess dietary Ca can cause poor absorption of many essential minerals (Cufadar *et al.*, 2011). But-high contents of Ca and Mg in water are known to reduce livestock performance. In addition, prolonged exposure to water that is high in Mg might reduce available Ca in body pools, which could affect eggshell and bone quality (Yenice *et al.*, 2015). Ariyamuni (2015) demonstrated that pH (8.2) of water affected hen production negatively. But the effects on poultry performance, including laying hens, of high levels of minerals in the water are not well documented. Thus, there is a need to conduct studies that reveal the effects of mineral supplementation in water on poultry performance. But there are apparently few established guidelines to assess the quality of drinking water that are based on experimentation on poultry (Blake *et al.*, 2019). Those that do exist are based on human standards and on recommendations for other livestock species. However, the use of human standards to evaluate animal drinking water may be inappropriate, since animals can tolerate higher levels of minerals and their ability to endure dissolved substances may be different from that of human beings (Youssef *et al.*, 2009). Because of the dearth of scientific information on the mineral effects of water on egg quality and production, this study was planned to fill these knowledge gaps in laying hen nutrition. Thus,

supplementation of Ca and Mg was hypothesized to affect the egg quality and production performance of hens. In this study, Ca and Mg were supplied in drinking water instead of in feed because water is consumed at a rate that is 1.6 - 2 times higher than feed. In addition, water is used to transport nutrients and for chemical and enzymatic reactions, which could make it a better carrier for the supplement. Therefore, the effects on production performance and egg quality of various levels of Ca and Mg in drinking water were assessed. Egg weight, eggshell crack, yolk colour, yolk height, albumen height, and Haugh unit were investigated. The study provided insights into the supplementation of Ca and Mg to improve the egg quality of hens, which could be used as a reference for the feed management of other birds.

## Materials and Methods

Calcium and Mg were provided by a commercial company (Yinchuan, Ningxia). These minerals had been extracted from deep ocean water pumped from a depth of 510 m in the South China Sea, according to the supplier. A total of 384 (62-week-old) Hy-line Brown laying hens were used in the six-week feeding trial. The birds were randomly divided into four treatments with eight replications of twelve birds. Hens were housed in individual cages in a windowless and environmentally controlled house with stable temperature (21 °C) and a light regime was set at 16 hours light and 8 hours dark. Birds were given ad-libitum access to feed and water throughout the trial. The four treatments consisted of CON, drinking water; T1, drinking water + 2 mg/L Ca + 250 mg/L Mg; T2, drinking water + 4 mg/L Ca + 510 mg/L Mg /10 L; and T3, drinking water + 5 mg/L Ca and 760 mg/L Mg. The experimental diets followed the recommendations of the Hy-Line Brown handbook and were provided as mash (Table 1).

**Table 1** Diet composition (as-fed basis) for Hy-line Brown laying hens

Ingredients	%	Analysed nutrient composition	
Corn	58.90	ME, kcal/kg	2,800
Soybean meal	27.22	Crude protein, %	17.20
Tallow	2.822	Fat, %	5.10
Tricalcium phosphate <sup>1</sup>	1.84	Calcium, %	3.9
Limestone	8.04	Total phosphorus, %	0.7
Vitamin premix <sup>2</sup>	0.12	Available phosphorus, %	0.45
Mineral premix <sup>3</sup>	0.10		
Salt	0.31		
DL-methionine (50%)	0.20		

<sup>1</sup>Tricalcium phosphate contains 32% calcium and 18% P phosphorus (National Research Council, 1994)

<sup>2</sup>Vitamin A: 125,000 IU, vitamin D3: 2,500 IU, vitamin E: 10 mg, vitamin K3: 2 mg, vitamin B1: 1 mg, vitamin B2: 5 mg, vitamin B6: 1 mg, vitamin B12: 15 mg, folic acid: 500 mg, niacin: 35,000 mg, Ca-Pantothenate: 10,000 mg, biotin: 50 mg per kg of diet

<sup>3</sup>Manganese: 8 mg, zinc: 60 mg, copper: 25 mg, iron: 40 mg, cobalt: 0.3 mg, iodine: 1.5 mg

Egg production and rate of broken eggs were measured weekly, whereas water consumption was recorded daily. Egg quality was checked weekly throughout the trial. A total of 40 eggs with single yolks and had shells that were free of visual defects and cracked shells were collected randomly at 17h00 per treatment (5 per replication, n = 40) per week and were used to determine egg quality at 20h00 on the same day. Egg weight, eggshell crack, yolk colour, yolk height, albumen height, and Haugh unit were tested with a digital egg tester (NABEL DET6000, Kyoto, Japan). Then eggshell thicknesses of the broader end, equatorial region and the narrow end were calculated with an eggshell thickness gauge. Finally, 300 defect-free eggs were collected randomly at 17h00 from each treatment. The eggs were stored in a refrigerated chamber (4 °C) and at room temperature (20 °C) for 0 (starting day), 1, 5, 10, and 15 days. Humidity was 55% to 60% for all treatments. The sampled eggs then were used to determine Haugh unit and albumen height at 20h00 on days 0, 1, 5, 10, and 15 with a digital egg tester (NABEL, DET6000, Japan). All measurements were recorded each week to investigate the supplementation effects of Ca and Mg and to optimize the duration of supplementation.

Data were analysed with a completely randomized design using mixed procedures of SAS (SAS Institute, Inc., Cary, North Carolina, USA) to quantify the linear and quadratic effects using polynomial regression. Effects with a probability level less than 0.05 were considered statistically significant, whereas  $0.05 \leq P < 0.10$  was regarded as indicating a trend.

## Results and Discussion

There was a linear decrease ( $P = 0.04$ ) in water intake in week 1 with increasing levels of mineral supplementation of the drinking water (Table 2). However, no significant ( $P > 0.05$ ) change in water intake was observed with the addition of Ca and Mg in the subsequent weeks.

**Table 2** Effects of supplementation of calcium and magnesium in drinking water on water intake in laying hens

Week	Treatments				SE	P-value	
	Control	T1	T2	T3		Linear	Quadratic
1	203	201	201	199	1.17	0.04	0.92
2	199	201	201	200	0.88	0.90	0.28
3	199	199	199	198	1.07	0.51	0.32
4	197	198	199	199	0.76	0.06	0.34
5	201	198	199	198	1.18	0.32	0.54
6	199	199	199	198	0.82	0.39	0.46
7	199	199	199	198	0.82	0.39	0.46

T1: 2 mg/L Ca + 250 mg/L Mg; T2: 4 mg/L Ca + 510 mg/L Mg /10 L; T3: 5 mg/L Ca + 760 mg/L Mg

The effects of higher levels of Ca and Mg and other minerals in the water should be assessed to find a maximum tolerable level for hens, at which performance and egg quality are not affected (Table 3). Likewise, Akbar *et al.* (1983) noted no change in egg weight, eggshell thickness, or egg breaking strength when laying hens were provided drinking water that contained 625 ppm  $MgSO_4$ , 1250 ppm  $MgSO_4$ , and 625 ppm  $MgSO_4$  plus 1417 ppm calcium sulphate. The decreased rate of broken eggs in the current study was attributed to improved eggshell thickness and eggshell strength.

**Table 3** Effects of supplementation of calcium and magnesium in drinking water on egg production, rate of broken eggs, and egg weight in laying hens<sup>1</sup>

	Treatments				SE	P-value	
	Control	T1	T2	T3		Linear	Quadratic
Egg production, %							
Week 1	85.0	85.4	85.6	85.9	1.80	0.72	0.97
Week 2	85.1	85.3	85.7	85.9	2.61	0.81	0.99
Week 3	85.0	85.4	85.6	85.7	1.36	0.64	0.87
Week 4	84.8	85.3	85.4	85.6	1.62	0.74	0.93
Week 5	84.5	85.0	85.4	85.7	1.94	0.65	0.97
Week 6	84.1	84.8	85.0	85.3	2.73	0.73	0.94
Broken egg rate, %							
Week 1	0	0.2	0.2	0.2	0.12	0.5	0.5
Week 2	0.3	0.0	0.2	0.2	0.25	0.63	0.29
Week 3	0.4	0.0	0.2	0.2	0.21	0.63	0.29
Week 4	0.5	0.0	0.0	0.0	0.12	0.00	0.01
Week 5	0.2	0.5	0.2	0.4	0.21	0.87	0.71
Week 6	0.3	0.2	0.3	0.0	0.35	0.47	0.74
Egg Weight							
Start	64.55	65.23	65.48	65.57	0.83	0.39	0.74
Week 1	65.18	65.34	65.31	65.48	0.98	0.84	0.99
Week 2	65.11	65.14	65.28	65.46	0.97	0.79	0.94
Week 3	64.79	65.03	65.11	65.37	1.06	0.69	0.99
Week 4	64.72	65.03	65.23	65.3	1.05	0.67	0.91
Week 5	65.04	65.15	65.29	65.38	1.04	0.8	0.99
Week 6	64.96	65.17	65.23	65.38	1.08	0.78	0.98

<sup>1</sup> Values of means represent 8 replications per treatment and 12 birds per replication  
T1: 2 mg/L Ca + 250 mg/L Mg, T2: 4 mg/L Ca + 510 mg/L Mg /10 L, T3: 5 mg/L Ca + 760 mg/L Mg

No significant differences were noted in egg production, egg weight and internal egg quality (eggshell colour, Haugh unit, albumen height, and yolk colour) on the starting day and in weeks 1, 2, 3, 4, 5, and 6 with increasing the Ca and Mg levels in the drinking water ( $P > 0.05$ ) (Table 4).

**Table 4** Effect of supplementation of calcium and magnesium in drinking water on egg quality in laying hens<sup>1</sup>

	Treatments				SE	P-value	
	Control	T1	T2	T3		Linear	Quadratic
Shell colour							
Start	10.0	10.3	10.3	10.4	0.30	0.37	0.72
Week 1	10.1	10.3	10.4	10.4	0.26	0.37	0.80
Week 2	10.2	10.3	10.2	10.7	0.30	0.24	0.48
Week 3	10.4	10.2	10.7	10.8	0.30	0.27	0.62
Week 4	10.3	10.1	10.2	10.9	0.32	0.18	0.18
Week 5	10.4	10.0	10.6	10.6	0.29	0.37	0.39
Week 6	10.5	10.2	10.6	10.7	0.31	0.50	0.59
Eggshell strength, kg/cm <sup>2</sup>							
Start	3.838	3.848	3.865	3.875	0.02	0.24	0.97
Week 1	3.812	3.833	3.876	3.884	0.03	0.06	0.82
Week 2	3.790	3.869	3.878	3.884	0.03	0.01	0.15
Week 3	3.792	3.818	3.827	3.833	0.03	0.23	0.69
Week 4	3.814	3.817	3.824	3.852	0.03	0.29	0.62
Week 5	3.769	3.816	3.836	3.858	0.03	0.01	0.63
Week 6	3.782	3.827	3.831	3.869	0.03	0.03	0.88
Eggshell thickness, mm							
Start	46.7	46.4	46.8	46.8	0.50	0.96	0.71
Week 1	46.9	46.9	46.6	46.5	0.49	0.43	1.00
Week 2	47.6	47.7	47.0	46.5	0.50	0.08	0.44
Week 3	45.9	46.2	46.7	46.8	0.50	0.17	0.82
Week 4	46.2	46.4	46.4	46.7	0.50	0.64	0.85
Week 5	46.3	46.6	46.8	46.9	0.51	0.35	0.87
Week 6	45.4	46.4	46.7	47.3	0.5	0.02	0.60

<sup>1</sup>Values of means represent 8 replications per treatment and 12 birds per replication

T1: 2 mg/L Ca + 250 mg/L Mg, T2: 4 mg/L Ca + 510 mg/L Mg /10 L, T3: 5 mg/L Ca + 760 mg/L Mg

Minerals in drinking water can change the quality of the eggshell. A number of studies found a harmful influence on eggshell quality (West *et al.*, 1980; Wang *et al.*, 2020) of high levels of sodium chloride (NaCl) in drinking water, but studies on the effects of minerals such as Ca and Mg in water on hen performance and egg quality are limited. In addition, results of studies of high levels of minerals in the diet or water for laying hens have differed. For example, Kim *et al.* (2013) found that the addition of 500 mg/kg Mg to the diet for laying hens was sufficient to improve eggshell thickness and subsequently reduce the proportion of breakages. Supplementation of Mg above 5 g/kg, at 4.7 g/kg, and at 3 g/kg increased the eggshell quality in laying hens (National Research Council 1994), but an excessive amount (2380 mg/kg) did not have a disadvantageous effect on egg production. In addition, Stilborn *et al.* (1989) suggested that the addition of 2500 mg/kg Mg to the practical diet for layers did not improve their eggshell quality (Ahn *et al.*, 1997).

However, adding treatments of Ca and Mg to the drinking water raised eggshell strength linearly (weeks 2, 5, and 6) ( $P=0.01$ , 0.01, 0.03, respectively), increased eggshell thickness (week 6) ( $P=0.02$ ), and decreased the broken egg rate quadratically (week 4) ( $P=0.01$ ) (Table 5).

**Table 5** Effect of supplementation of calcium and magnesium to drinking water for laying hens on interior egg quality<sup>1</sup>

	Treatments				SEM	P-value	
	CON	T1	T2	T3		Linear	Quadratic
Haugh Unit							
Start	90.3	90.5	90.7	90.9	0.78	0.57	0.99
Week 1	90.2	90.4	90.8	91.5	0.91	0.29	0.73
Week 2	90.3	90.8	90.8	91.6	0.95	0.38	0.88
Week 3	89.4	90.3	90.3	91.3	1.35	0.35	0.98
Week 4	89.7	90.0	90.6	91.7	1.3	0.28	0.74
Week 5	89.4	90.8	91.4	91.7	1.34	0.22	0.68
Week 6	89.0	90.2	90.5	91.0	1.37	0.31	0.79
Albumen height, mm							
Start	8.2	8.4	8.5	8.4	0.21	0.39	0.71
Week 1	7.9	8.3	8.5	8.4	0.21	0.07	0.18
Week 2	8.3	8.2	8.0	8.1	0.24	0.43	0.57
Week 3	8.3	8.5	8.5	8.7	0.24	0.25	0.95
Week 4	8.3	8.4	8.5	8.8	0.25	0.20	0.77
Week 5	8.3	8.5	8.7	8.8	0.24	0.15	0.63
Week 6	8.2	8.4	8.6	8.6	0.24	0.24	0.75
Yolk colour							
Start	8.2	8.2	8.4	8.2	0.26	0.89	0.65
Week 1	8.3	8.4	8.2	8.6	0.28	0.55	0.72
Week 2	8.2	8.3	8.3	8.1	0.26	0.82	0.53
Week 3	7.9	8.4	8.6	8.2	0.36	0.41	0.2
Week 4	7.7	8.4	8.1	8.0	0.36	0.72	0.29
Week 5	8.0	8.0	8.3	8.0	0.35	0.83	0.77
Week 6	7.9	7.8	8.1	8.2	0.37	0.43	0.82

<sup>1</sup>Values of means represent 8 replications per treatment and 12 birds per replication

T1: 2 mg/L Ca + 250 mg/L Mg, T2: 4 mg/L Ca + 510 mg/L Mg /10 L, T3: 5 mg/L Ca + 760 mg/L Mg

Haugh unit and albumen height were not affected by increasing the concentration of the Ca and Mg in the drinking water ( $P > 0.05$ ) after storing the eggs for 1, 5, 10, and 15 days (Table 6).

Eggs contain high biological value protein and essential minerals (Hawley *et al.*, 1946). The diet that is provided to laying hens has a crucial effect on the quality of eggs (Kowalska *et al.*, 2021). The quality of water influences the quality of eggs, even if a balanced diet is provided (Damron & Flunker, 1995). Although water does not supply energy, it is necessary for various metabolic functions. Increased levels of minerals can be undesirable to birds, and reduce performance (Yoselewitz *et al.*, 2008). In agreement with the current results, Yoselewitz *et al.* (2012) reported that water consumption was lower at 625, 1250, and 1417 ppm magnesium sulfate (MgSO<sub>4</sub>) than that of control. Pourreza *et al.* (1994) found a parallel effect of high MgSO<sub>4</sub> in the drinking water on its consumption of laying hens. In addition, Sus and Balnave (1975) found that daily water consumption was significantly lower when Ca was supplied at 3.5% and 2.5% in the diet and at 0.2% in the drinking water. The decrease in water intake in the first week of experiment could be because of the change in taste when Ca and Mg were added. However, after the first week, laying hens might adapt to the change. No significant impact was noticed in continued weeks (Table 2).

**Table 6** Effect on Haugh unit and albumen height<sup>1</sup> of supplementation of calcium and magnesium in drinking water for laying hens

	Treatments				SE	P-value	
	Control	T1	T2	T3		Linear	Quadratic
Haugh Unit							
Start	89.60	90.25	90.67	92.32	2.25	0.41	0.83
Day 1	88.27	89.42	90.57	91.48	2.34	0.31	0.93
Day 5	87.53	88.03	88.58	90.58	2.49	0.39	0.77
Day 10	83.20	84.45	84.70	85.80	1.24	0.16	0.95
Day 15	80.83	81.27	82.33	83.63	0.88	0.27	0.63
Albumen height, mm							
Start	8.13	8.23	8.28	9.38	0.33	0.02	0.15
Day 1	7.75	8.58	8.75	8.40	0.50	0.35	0.25
Day 5	7.93	8.22	8.48	8.63	0.42	0.22	0.88
Day 10	7.97	8.13	8.37	8.43	0.29	0.22	0.86
Day 15	7.82	8.07	8.15	8.25	0.46	0.56	0.91

<sup>1</sup>Values of means represent 8 replications per treatment and 12 birds per replication

T1: 2 mg/L Ca + 250 mg/L Mg, T2: 4 mg/L Ca + 510 mg/L Mg /10 L, T3: 5 mg/L Ca + 760 mg/L Mg

Albumen height, albumen percentage and yolk percentage were not affected when laying hens were provided drinking water with 625 ppm MgSO<sub>4</sub>, 1250 ppm MgSO<sub>4</sub>, and 625 ppm MgSO<sub>4</sub> plus 1417 ppm calcium sulphate in the study of Akbar *et al.* (1983). According to Keener *et al.* (2006), the Haugh unit is a measure of albumen quality and therefore of the freshness of the egg, whereas Silversides *et al.* (1993) proposed that albumen height should be measured to determine egg quality. Various factors affect Haugh units, including storage time, temperature, age of hen, strain, and nutrition (Lapao *et al.*, 1999). Temperature and storage time appear to be crucial factors that affect albumen quality and Haugh unit (Jones *et al.*, 2001). Eggshell thickness was related negatively to eggshell water conductance (Samli *et al.*, 2005).

## Conclusion

The addition of up to 5 mg/L Ca and 760 mg/L Mg (T3) to the drinking water improved eggshell thickness and eggshell strength without unfavourable consequences to egg quality and egg production of laying hens.

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## Authors' Contributions

XJY, AR and HSH conceived and designed the experiments. XJY, AR, RY and RWA analysed the data and drafted the manuscript. YNG, AR, HMI and HSH performed experiments and acquired data. YNG, RZA, KH, MAR and AR revised the manuscript. All authors read and approved the final manuscript.

## Conflict of Interest Declaration

There is no conflict of interest for this study.

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