

Effects of Magnetized Water on *In-Vitro* Calcium Carbonate Solubility and Eggshell Breaking Strength

E. Darsi¹, H. Kermanshahi^{1*}, H. Nassiri Moghaddam¹, A. Golian¹, and M. Gholizadeh²

ABSTRACT

This study was conducted with two consequential experiments to investigate the effect of magnetized water on *in-vitro* limestone solubility and eggshell breaking strength in laying hens. The first experiment was conducted to investigate the effect of magnetized water and particle size on *in-vitro* limestone solubility. Three particle sizes including small particles, less than 0.125 mm; large particles, 2-4 mm and normal mix, 0.045-4.00 mm were tested. Magnetized water was generated by magnetizer of 0.65 Tesla magnetic fields. Scanning Electron Microscopy images (SEMi) were used to study the morphology of limestone crystals after dissolution in magnetized and tap water. Limestone solubility was measured by 0.2N HCl solution. The second *in-vivo* experiment was conducted to study the effect of magnetized water and dietary calcium on breaking strength of eggshell. Three dietary levels of calcium and phosphorus (normal, 10 and 20% reduced Ca and available P.) and two types of water (tap water and magnetized water) were used in Hy-line laying hens at 32 weeks of age. Breaking strength of the normal (safe and sound) eggs was measured with an Instron testing machine. The solubility of large limestone particles was less than those of small particles. Magnetically treated water did not change *in-vitro* limestone solubility but changed the morphology of limestone crystals. Precipitated limestone crystals in magnetized water tended to be larger and more uniform in size than those in tap water. Reducing dietary levels of Ca and P had no significant effect on egg breaking strength at 36 weeks of age. Magnetized water was able to numerically increase strength of the eggs. Therefore, based on the SEMi and the observed changes in crystalline structure of dissolved (exposed to water) precipitated limestone and observed changes in breaking strength of the eggs, it is hypothesized that magnetized water may have the potential to change the limestone availability and consequently egg strength in laying hens.

Keywords: Limestone solubility, Magnetized water, Particle size, Scanning electron microscope.

INTRODUCTION

A new technique patented by researchers, consists of exposing water to a magnetic field, pointed to the possibility of changes in water molecules arrangement (Gehr *et al.*, 1995). The changes in the structure of water through exposure to a magnetic field have various applications, such as increase in the

compressive strength of concrete, precipitation process of calcium carbonate (Su and Wu, 2003; Fathi *et al.*, 2006), and reduction of the corrosion rate of steel (Bikul'chuyus *et al.*, 2003).

Literature survey indicates that many authors were interested in the morphology of precipitated calcium carbonate. It is reported that magnetic field actually affects the morphology of precipitated calcium

¹ Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.

* Corresponding author; e-mail: kermansh@um.ac.ir

² Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.



carbonate (Parsons *et al.*, 1997; Coey and Cass, 2000). Although presence of some magnetic field has effects on the properties of precipitated calcium carbonate and seems to be now well documented (Gehr *et al.*, 1995; Parson *et al.*, 1997; A-Barrett and Parsons, 1998), this issue is still a controversial one (Baker *et al.*, 1997). Even though, some authors (Parsons *et al.*, 1997) claim that magnetic treatment may reduce the scale formation in a recirculating system by 50%, others (Busch *et al.*, 1997) have found only minor or no effect of the field.

The magnetic effect can be broadly divided into physical and chemical mechanisms. The chemical mechanisms include a modification of crystal liquid interface including the changes in crystallite nucleation (A-Barrett and Parsons, 1998). Several authors reported that larger crystallites were formed as a result of magnetic pre-treatment and the deposit was more porous. This procedure inhibits precipitation of the mineral deposits on the inside walls of pipes and other pieces of equipment (Baker *et al.*, 1997; Gryta, 2011). These particles are carried away by the water flow and can be eliminated by removing or filtering the resulting calcareous mud (A-Barrett and Parsons, 1998; Baker *et al.*, 1997). Gryta (2011) concluded that the application of magnetic water treatment for the feed treatment during membrane distillation process did not only decrease the amount of forming precipitate, but also affected its morphology (Gryta, 2011). The formed scaling layer was more porous made by larger crystallites. However, the degree of scaling reduction was too low and the precipitate was still forming on the membrane surfaces.

The physical mechanism includes an alteration in some physical properties of water such as surface tension and size of cluster (Toledo *et al.*, 2008; Amiri and Dadkhah, 2006) that may improve the mineral solubility of water. It has been shown that magnetic treatments of water prevents the formation of, as well as lead to the removal of mineral scale deposits from

pipelines and equipment in industry, by increasing the solubility of calcium salts (Chynoweth, 1985; Skeldon, 1990). Magnetization for improvement of minerals solubility has also been used to prevent and remove mineral deposits in milking machine pipelines (Andersson and Knudsen, 1983). It has been suggested that minerals in aqueous solutions are modified by the magnetization process passing more readily through biological membranes (Lin, 1990).

Hen eggs as necessary foodstuff deserve an increased attention, mainly their eggshells. The eggshell microstructure is determined by genetic, physiological and external factors. Eggshell microstructural characteristics can inform us about biological and physicochemical processes affecting its formation (Rodriguez-Navarro, 2007). For eggshell formation during laying periods, the hen needs to take in 2.0–2.2 g of calcium from the feed and for this reason it is necessary to provide 3.5–4.30% Ca (Tullet, 1987) in the feed. Adequate dietary levels of calcium have to be provided during rearing, transition and laying periods but a further increase in the calcium level or the use of Ca step-up phase feeding system are poorly efficient (Nys, 1999). A deficiency of calcium is generally recognized to cause a decline in egg production and a reduction in the thickness of the eggshell.

The solubility of limestone as a Ca source in laying hen gastro-intestinal tract may affect calcium absorption and results in a higher eggshell quality. Therefore, any new findings about limestone solubility would have the potential to improve eggshell quality in commercial egg industry.

Water treatment by magnetic field is still a controversial subject, because the reported results have low reproducibility and little consistence. It can be concluded that more experimental evidences are needed to verify the controversial hypotheses dealing with mechanisms of magnetic field. The purpose of this study was to investigate the effect of magnetic water treatment on *in-vitro* limestone solubility and *in-vivo* eggshell quality.

MATERIALS AND METHODS

At the first experiment in a 2×3 factorial arrangement of completely randomized design, the effect of two water sources, magnetic water treatment and tap water and three limestone particle sizes (small, large and normal mix) on *in-vitro* limestone solubility were examined. Briefly, three samples of 2 kg were each taken from an available source of limestone. Every sample was sifted by Model AS-200 digit vibratory sieve shaker (RETSCH Company, Haan, near Düsseldorf, Germany) to separate the particle sizes of 0.042, 0.063, 0.125, 0.25, 1.00, 2.00, and 4.00 mm. A sample of the limestone was placed on the top screen and allowed to shake on sieve shaker for 5 minutes and then particle sizes were collected from each screen. The percent of each particle size was calculated by dividing the weight of the particles collected at the respective screen size to the total weight of the sample.

Duplicate sub-samples of the small particles (< 0.25 mm), large particles (2.00-4.00 mm) and unsifted mix (normal mix) were used to study the effect of magnetized and tap water on limestone solubility. The percent of weight loss method was used to measure *in-vitro* solubility of the sub limestone samples as described by Zhang and Coon (1997). A beaker of 600 ml volume was filled with 200 ml of a 0.2N HCl solution to act as a solvent for each of the limestone samples. Tap water was magnetized with a 0.65 Tesla permanent magnet. Tap water and magnetized water were used to prepare a 0.2N HCl of either tap or magnetized solutions. The 0.2N HCl solution was warmed up for 15 minutes at 42°C in a water bath until the solution temperature reached a constant. A sub-limestone sample (2.0 g) was poured into the solution and allowed to react for 10 minutes. After the reaction time, limestone was filtered with a GmbH filter paper (No.

006). The precipitated limestone was oven dried in 60°C and limestone weight loss was used to calculate *in-vitro* solubility (Zhang and Coon, 1997). After the measurement of *in-vitro* solubility of the sub-limestone samples, the morphology of retained limestone precipitate was studied using LEO 1450VP Scanning Electron Microscopy (Carl ZEISS Company, Jena, Thuringia, Germany).

In an *in-vivo* study with a 2×3 factorial arrangement, three dietary levels of calcium and phosphorus (4.20% Ca, and 0.48% available P. as normal level; 3.78% Ca, and 0.43% available P. as 10% lowered level; and 3.36% Ca, and 0.38% available P. as 20% lowered level) and two types of water (magnetized and tap water) were used in laying hens to find out the effect of magnetized water on eggshell breaking strength. The experiment was carried out on 360 Hy-line W-36 laying hens at 32 weeks of age, allocated to 30 experimental units of 5 replications from 6 treatments. Each experimental unit contained 3 consecutive cages (with dimensions of 45×45 cm, equaling 1,600 cm² of floor space) of 4 hens in each cage. Feed and water provided ad-libitum. Birds were exposed to 16 hours of light/day during the experiment. Diets contained normal, 10 or 20% lowered levels of Ca and available P. (Table 1). Magnetized water was produced by a commercial magnet namely AGUA CORRECT with 0.65 tesla (6500 Gauss) magnetic field. At 36 weeks of age, 5 normal eggs from each treatment were collected for the measurement of eggshell breaking strength. Breaking strength from blunt to sharp end of each egg was measured with an Instron testing machine, model H5K-S UTM. All data were analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS, 2004). Tukey's Studentized Range (HSD) test was used to compare treatment means. The *P* values of 0.05 or less were considered significant (*P*< 0.05).

**Table 1.** Ingredient composition of experimental diets.

Ingredients (%)	Ca level (%)		
	4.20%	3.78	3.36
Corn	62.00	64.75	67.50
Soybean meal	22.48	21.91	21.34
Di-calcium Phosphate	1.85	1.57	1.28
Limestone	9.86	8.62	7.97
Vitamin premix ^a	0.25	0.25	0.25
Mineral premix ^b	0.25	0.25	0.25
Salt	0.33	0.33	0.33
Vegetable Oil	2.67	1.70	0.73
DL-Methionine	0.26	0.26	0.25
L-Lysine	0.05	0.07	0.08
Calculated analysis (%)			
Calcium	4.20	3.78	3.36
ME (kcal kg ⁻¹)	2844	2844	2844
Crude protein	15.50	15.50	15.50
Crude fat	1.48	2.59	2.68
Crude fiber	3.18	3.21	3.24
Available P.	0.48	0.43	0.38
Linoleic acid	2.10	1.84	1.60
Sodium	0.18	0.18	0.18
Arginine	0.97	0.97	0.96
L-Lysine	0.82	0.82	0.82
Methionine	0.51	0.51	0.51
Threonine	0.76	0.76	0.76
Tryptophan	0.20	0.30	0.20

^a Vitamin premix provided per kilogram of diet: Vitamin A (retinyl acetate), 8,800 IU; cholecalciferol, 2,200 IU; DL-tocopheryl acetate, 11 IU; menadione sodium bisulphate, 2.2 mg; riboflavin, 4.4 mg; D-calcium pantothenate, 8.8 mg; nicotinic acid, 44 mg; pyridoxine hydrochloride, 2.2 mg; d-biotin, 0.11 mg; thiamine hydrochloride, 2.5 mg; ethoxyquin, 125 mg.

^b Mineral premix provided per kilogram of diet: MnSO₄·H₂O, 185 mg; ZnO, 62 mg; FeSO₄·7H₂O, 149 mg; CuSO₄·5H₂O, 19.6 mg; KI, 1.4 mg; Na₂SeO₃, 0.22 mg.

RESULTS

The sieve analysis results from the three samples of a limestone source are shown in Table 2. The particle size distribution of three samples taken from a limestone source were found to be very similar, all had about 86% of 2-4 mm particle size indicating the high uniformity in that source of calcium carbonate used in this study. Therefore, every sample was used as one replicate to determine *in-vitro* limestone solubility.

The effects of particle size and magnetized water on *in-vitro* limestone solubility are

shown in Table 3. The *in-vitro* solubility of large particle (2-4 mm) limestone source was less than those of small particle sizes. The magnetically treated water did not affect *in-vitro* limestone solubility. However, an interaction effect between particle size and type of water was observed and limestone solubility in both magnetized water and top water were decreased by lowering the particle size of limestone.

In this study the SEMi revealed that the dissolution process has dramatically changed the shapes of limestone crystals. The crystals in undissolved limestone (unexposed to water) were granular and equant (used to describe rounded as well as

Table 2. Percent of particle size distribution of a limestone source.

Particle size rang (mm)	Ca source			Means
	Sample 1	Sample 2	Sample 3	
> 4.00	0.94	0.87	1.02	0.94
2.00-4.00	84.33	86.17	88.02	86.17
1.00-2.00	6.14	4.84	4.61	5.19
0.5-1.00	1.89	0.91	1.38	1.39
0.25-0.50	1.01	0.55	0.75	0.77
0.125-0.25	4.15	2.85	1.49	2.83
0.063-0.125	1.42	3.38	2.69	2.49
0.045-0.063	0.07	0.40	0.00	0.15

Table3. Effect of particle size and magnetized water on *in-vitro* limestone solubility.

Treatment	Solubility (%)	
Main effects		
Particle size (mm)		
Small ^a		90.196 ^a
Large ^b		62.754 ^b
Normal mix ^c		61.579 ^b
SEM ^d		2.3800
P-value		< 0.0001
Type of water		
Tap Water		70.86
Magnetized water		72.23
SEM		1.590
P-value		0.4488
Interaction (Type of water×Particle size)		
Particle size (mm)	Type of water	Solubility (%)
Small	TW ^e	89.32 ^a
Large	TW	64.82 ^b
Normal mix	TW	62.53 ^b
Small	MW ^f	91.06 ^a
Large	MW	60.63 ^b
Mix	MW	60.82 ^b
SEM		4.910
P-value		< 0.0001

^a Small particle size (particle less than 0.125 mm); ^b Large particle size (particle between 2-4 mm); ^c Normal mix particle size (unsifted limestone); ^d Standard Error of the Mean (SEM); ^e Tap Water, ^f Magnetized Water.

^{a-b} Values within columns without a common letter differ significantly ($P \leq 0.01$).

angular, spherical or round crystals) with big spaces among crystals (Figure 1-a), but the crystals in dissolved limestone were completely thin and flattened and there was little spaces among crystals (Figure 1-b). The magnetized water seems to affect the dissolved crystallites morphology and visual differences were noticed (SEM observations) in the shape

of limestone crystals. The crystals of dissolved limestone in magnetized water tended to be larger than those in tap water, the spaces among limestone crystals disappeared, and the crystals had a great uniformity in size (Figure 1-c).

The effects of magnetized water and dietary calcium on eggshell breaking strength are

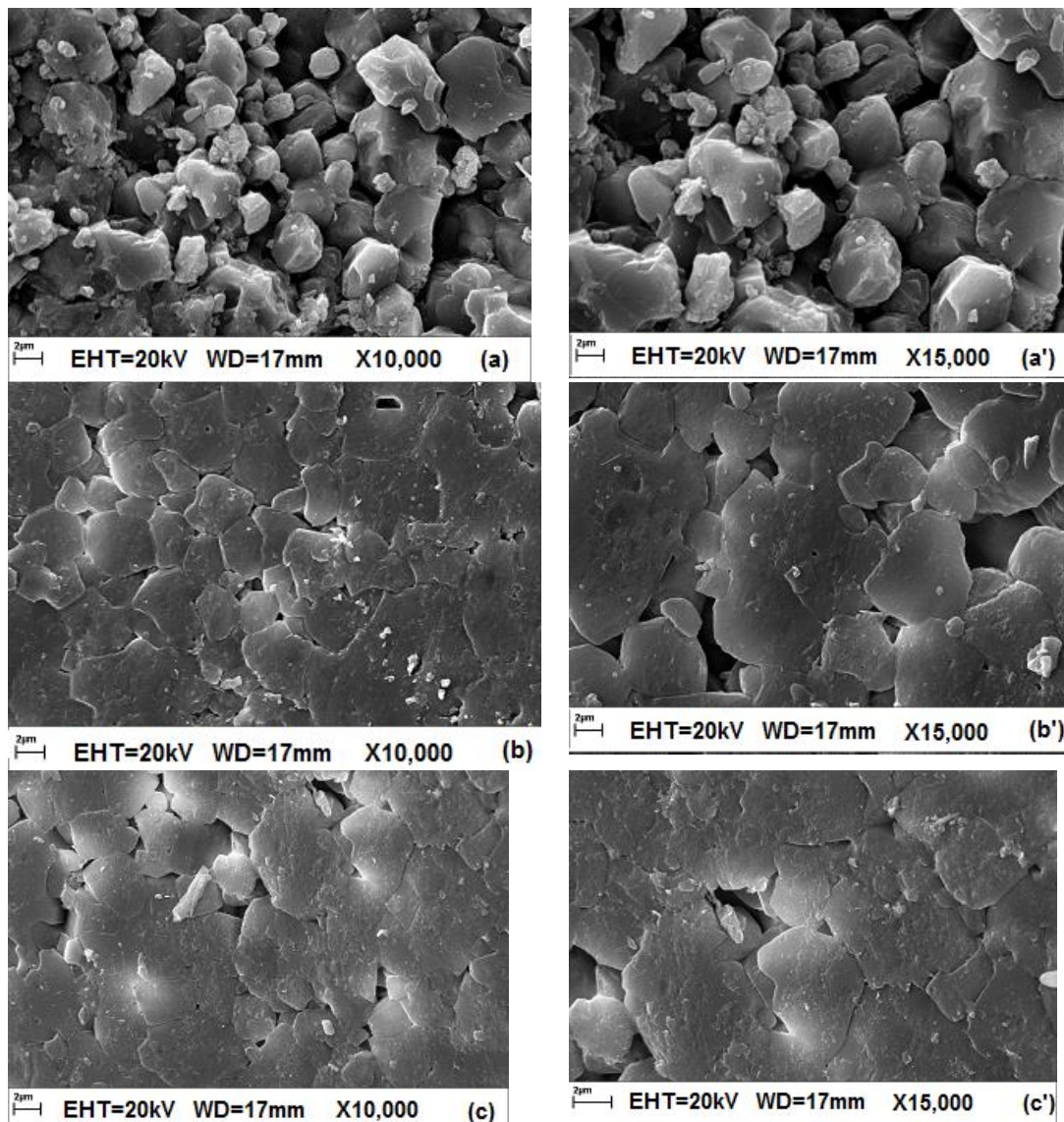


Figure 1. Scanning Electron Microscopy images (SEMi) from undissolved (unexposed) limestone in water (a: X10,000; a': X15,000), SEMi from dissolved (exposed) limestone in tap water (b: X10,000; b': X15,000), SEMi from dissolved limestone in magnetized water (c: X10,000; c': X15,000),

shown in Table 4. The results showed that by reducing dietary levels of calcium, breaking strength was numerically decreased. Magnetized water exhibited no significant increase in strength of the eggs.

DISCUSSION

In our study, the *in-vitro* solubility of large particle limestone source was less than those of small particle sizes that might be due to

the surface area of large particle limestone which is lower than that of small particles, resulting in a reduced reactive surface for HCl acid (De Witt *et al.*, 2006). Other studies confirmed that the larger particles (> 2.00 mm) were less solubilized than those of small particle sizes (De Witt *et al.*, 2006; Saunders-Blades, 2009). Magnetized water did not affect *in-vitro* limestone solubility. Based on the results of some studies the magnetized water could increase the solubility of minerals and consequently

Table 4. Effects of dietary Ca level and magnetized water on egg shell breaking strength.

Treatment	Breaking strength of egg (n)	
Main Effects		
Dietary calcium (%)		
4.20		
3.78		33.333
3.36		32.167
SEM ^a		30.100
P-value		2.81
		0.687
Type of water		
Magnetized water		32.781
Tap water		30.628
SEM		2.38
P-value		0.509
Interaction (Calcium level×Type of water)		
4.20	MW ^b	36.867
3.78	MW	28.806
3.36	MW	33.467
4.20	TW ^c	28.917
3.78	TW	37.208
3.36	TW	26.733
	SEM	3.72
	P-value	0.271

^a Standard Error of the Mean (SEM); ^b Magnetized Water, ^c Tap Water.

decrease the formation of mineral scale deposits on pipelines and equipments in the industry (Chynoweth, 1985; Andersson and Knudsen, 1983). It is well-known that the water molecules are in the form of clusters. Clusters of water molecules come in many sizes, depending on the number of water molecules involved. It has been shown by Toledo *et al.* (2008), when magnetic field applied to normal water, competition among the different hydrogen bonds and networks (intra- and intermolecular) gives rise to the weakening of the hydrogen bonds intra-cluster forming smaller cluster with stronger inter cluster hydrogen bonds (Toledo *et al.*, 2008). The result may provide maximum hydration with less water. They concluded that the *in-vitro* solubility of limestone could increase by magnetized water. In some studies it was suggested that application of magnetic water treatment affects the morphology of calcium carbonate precipitate. Calcium carbonate has three

anhydrous crystalline polymorphs including vaterite, aragonite, and calcite. The particles of calcium carbonate crystallized in a magnetic field are mainly consisted of a mixture of aragonite and vaterite. However, significant amounts of calcite were also observed (A-Barrett and Parsons, 1998; Baker *et al.*, 1997). Calcite is usually associated with hard scale whereas aragonite and vaterite give rise to a softer type of scale that is easily removed. Several reports revealed that magnetic water treatment would tend to reduce the nucleation rate and to accelerate the crystal growth (A-Barrett and Parsons, 1998; Baker *et al.*, 1997). The formed scaling layer had larger and more porous crystallites (Gryta, 2011; Chibowski, 2003a; Chibowski, 2003b) and can be carried away by water flow. In this study no effect of magnetized water on limestone solubility was observed. However, an interaction effect between particle size and type of water was observed. This interaction



could be due to better hydration of smaller sizes of limestone when faced with magnetized water. It is possible that the produced magnetized field has not been so strong to modify the solubility of limestone particles in larger sizes.

Most of the studies on the effects of nutrition on eggshell quality in laying hens have been focused on macro minerals (Ca, P) and vitamin D₃ (Korelesk and Swiathiewicz, 2004). Supplying the hen with an optimal Ca intake is the most crucial factor in order to ensure the proper calcification of the eggshell, but increasing the Ca level in the diet to above 3.6–3.8% usually has no beneficial effect on eggshell quality. The use of a particulate limestone as a source of Ca for layers has been shown to be more efficient in terms of the shell breaking strength (Chibowski, 2003a).

It is shown that *in-vivo* solubility of calcium particle sizes is reversibly related to its *in-vitro* solubility. A larger particle limestone with lower *in-vitro* solubility accumulates in the gizzard and produces high *in-vivo* solubility (Gehr *et al.*, 1995). Many studies with hens have shown that calcium sources with large particle size prolong the residence time of the particles in the stomach of poultry, favoring solubilization so that calcium ions are made available in sufficient quantities for absorption. Zhang and Coon (1997) noted that large particle size of limestone (> 0.8 mm) with lower *in-vitro* solubility (30 to 50%) was retained in the gizzard for a longer time, thereby increasing its *in-vivo* solubility to as much as 94% (Zhang and Coon, 1997). Rao and Roland (1989) showed that an increased proportion of calcium was solubilized from larger particles of limestone in the digestive system during a 25-hour period, providing greater availability of calcium ions for absorption (Rao and Roland, 1989).

It was indicated that residence time of Ca sources in the digestive system is an important factor for formation of egg with optimum shell quality. Therefore, the large limestone crystals could probably improve

the eggshell strength. The results in this study showed that by reducing dietary levels of calcium, breaking strength was numerically decreased. In a study on Bowans brown hens, reducing dietary level of Ca and P significantly decreased eggshell breaking strength (Koreleski and Swiathiewicz, 2004). Increasing dietary level of Ca had no beneficial effect on egg quality (Nys, 1999). The magnetized water almost increased breaking strength of the eggs. This effect can be attributed to larger limestone crystals and increased availability of Calcium in the gastrointestinal tract of the hens. These results are in agreement with those of Koreleski and Swiathiewicz (2004) who reported that larger particles of Ca supplements had generally an ameliorating effect on eggshell quality.

CONCLUSIONS

Under the conditions of this study it was confirmed that the flow of water through the magnetic field had a considerable effect on the morphology of CaCO₃ crystallites that remained during dissolution process. However, the results of *in-vitro* solubility tests showed the magnetized water had no significant effect on limestone solubility. However, solubility of calcium carbonates can be affected by its particle sizes both in *in-vitro* and *in-vivo* studies. Shell strength was greater in birds which consumed magnetized water. Based on the scanning electron microscopy images and the observed changes in crystalline structure of dissolved limestone and the effect of magnetized water on shell breaking strength of the eggs, it was hypothesized that the magnetized water may have a potential to improve availability of limestone and eggshell quality in the laying hens.

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تأثیر آب مغناطیسی بر حلالیت کربنات کلسیم در شرایط برون تنی و اثر آن بر استحکام تخم مرغ

ا. داری، ح. کرمانشاهی، ح. نصیری مقدم، ا. گلیان و م. قلی زاده

چکیده

این پژوهش در قالب دو آزمایش برای بررسی تأثیر استفاده از آب مغناطیسی بر حلالیت کربنات کلسیم در شرایط برون تنی و نیروی لازم برای شکست تخم مرغ انجام شد. آزمایش اول برای تعیین اثر آب مغناطیسی و اندازه ذرات کربنات کلسیم بر میزان حلالیت کربنات کلسیم در شرایط برون تنی انجام شد. اندازه ذرات کربنات کلسیم به سه گروه ذرات با اندازه کمتر از $0/125$ میلی‌متر، $2-4$ میلی‌متر و ترکیبی از این دو اندازه، تقسیم شدند. آب مغناطیسی از طریق مغناطیس کردن آب در یک میدان مغناطیسی با قدرت $0/65$ تسلا تولید شد. میزان حلالیت کربنات کلسیم با استفاده از محلول اسید کلریدریک $0/2$ نرمال اندازه گیری شد. برای مشخص شدن اثر آب مغناطیسی بر میزان حلالیت منبع کلسیمی، رقیق کردن اسید کلریدریک با آب آشامیدنی معمولی و آب مغناطیس شده انجام شد. پس از انجام مراحل حلالیت کربنات کلسیم، نمونه‌های کربنات کلسیم باقیمانده برای مشاهده شکل ظاهری مورد استفاده قرار گرفتند. شکل ظاهری ذرات با استفاده از میکروسکوپ الکترونی روبشی اندازه گیری شد. آزمایش دوم برای بررسی تأثیر نوع آب مصرفی و سطح کلسیم جیره بر استحکام تخم مرغ در مرغ‌های سویه هایلین انجام شد. در این آزمایش اثر دو نوع آب آشامیدنی (آب معمولی و آب مغناطیس شده به مدت چهار ساعت) و سه سطح کلسیم جیره (سطح کلسیم توصیه شده سویه تجاری هایلین دبلو-۳۶ و سطوح ۱۰ و ۲۰ درصد پایین تر از حد توصیه شده) بر قدرت شکست تخم مرغ-های تخمگذار در سن ۳۲ هفتگی بررسی گردید. نیروی لازم برای شکست تخم مرغ با استفاده از دستگاه اینسترون اندازه گیری شد. ذرات درشت کربنات کلسیم حلالیت کمتری نسبت به ذرات ریز داشتند. استفاده از آب مغناطیسی نتوانست حلالیت کربنات کلسیم در شرایط برون تنی را تحت تأثیر

قرار دهد اما شکل ظاهری ذرات کربنات کلسیم را تغییر داد. بلورهای سنگ اهک حل شده در آب مغناطیسی بزرگ‌تر و یکدست‌تر از بلورهای رسوب کرده در آب معمولی بودند. کاهش سطح کلسیم و فسفر جیره تاثیر معنی داری بر قدرت شکست تخم مرغ در ۳۶ هفتگی سن نداشت. آب مغناطیسی توانست قدرت و استحکام تخم مرغ را افزایش دهد. بنابراین بر مبنای تصاویر میکروسکوپ الکترونی و تغییرات مشاهده شده در شکل ظاهری ذرات کربنات کلسیم و نیروی شکست تخم مرغ، به نظر می‌رسد آب مغناطیسی می‌تواند میزان دسترسی کربنات کلسیم توسط طیور را افزایش دهد.