

Original research paper

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## THE INFLUENCE OF LIQUID CALCIUM SOURCE ON EGG SHELL QUALITY

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### Abstract

It is estimated that the world's population will enlarge by 25% by the middle of this century, resulting in the food production increase by at least 60%. Intensifying egg production is one of the most affordable solutions to provide animal protein. Among many other efforts to improve the quality of eggs, special attention is paid to attempts to provide a better endurance and strength of eggshell, due to the fact that the production of eggs with broken, cracked or soft shells incurs significant economic losses. One of the most important factors to achieve this goal is careful adjustment of calcium in the diet of laying hens, but the nutritional role of calcium is closely linked to that of phosphorus and the effect of vitamin D.

The aim of the experiment was to determine whether two different nutritional supplements of calcium, applied in drinking water of laying hens for two weeks have any significant influence on the shell egg quality. Statistically very significant difference in egg production was observed in the treatment with the product containing calcium and phosphorus. On the other hand, the product consisting of calcium and vitamin D3 had no beneficial impact on the egg parameters.

Based on the obtained results and literature data, it can be concluded that the amount and source of calcium in the diet of laying hens is a very complex and not fully solved issue. Therefore, especially keeping in mind the duration of the experiment, additional research is needed on this subject.

**Key words:** calcium, eggshell, laying hens, liquid supplement

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## UTICAJ TEČNOG DODATKA KALCIJUMA NA KVALITET LJUSKE JAJETA

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### Kratak sadržaj

Procenjuje se da će se svetska populacija uvećati za 25% do sredine ovog veka, te da će do tada i potreba za proizvodnjom hrane porasti za 60%. Intenziviranje proizvodnje jaja je jedno od najpristupačnijih rešenja za obezbeđivanje neophodnih proteina životinjskog porekla. Među mnogim drugim naporima da se poboljša kvalitet jaja, posebna pažnja se posvećuje nastojanju da se unapredi izdržljivost i jačina ljuske jajeta, zbog činjenice da je proizvodnja jaja sa slomljenom, napuklom ili mekanom ljuskom uzrok značajnih ekonomskih gubitaka. Jedan od najvažnijih faktora za postizanje ovog cilja je pažljivo balansiranje kalcijuma u ishrani koka nosilja, kao i izbor odgovarajućeg izvora mineralnih materija.

Cilj eksperimenta bio je da se utvrdi imaju li dva različita nutritivna dodatka kalcijuma, primenjeni u vodi za piće nosilja tokom dve nedelje, značajnog uticaja na kvalitet ljuske jaja. U grupi nosilja u čiju je vodu za piće dodat proizvod koji sadrži kalcijum i fosfor, u odnosu na kontrolnu grupu, uočene su statistički značajne razlike u broju proizvedenih jaja. S druge strane, proizvod koji se sastoji od kalcijuma i vitamina D3 nije imao uticaja na ispitivane parametre ljuske jaja.

Na osnovu dobijenih rezultata i podataka iz literature, može da se zaključi da je pitanje količine i izvora kalcijuma u ishrani nosilja veoma složen i ne u potpunosti rešen problem. Naročito imajući na umu dužinu trajanja eksperimenta, neophodna su dodatna istraživanja na ovu temu.

**Ključne reči:** kalcijum, ljuska jaja, nosilje, tečni dodatak

### INTRODUCTION

It is estimated that until 2050 the world's population will enlarge by 25%, and the demand for increase in food production will grow by at least 60% (FAOSTAT, 2013). Eggs constitute one of the most affordable source of animal protein available, so the number of laying flocks is rapidly increasing in developing countries. In Europe, the priority is to increase egg production by breed-

ing for increased persistency in lay and stability in egg quality so that the laying cycle of commercial flocks can be extended to 90–100 weeks (Bain et al., 2016).

A number of factors have an impact on the quality of eggs, such as breed and age of laying hens, inheritance, feed, the environmental conditions or diseases. Endurance and strength of eggshells require special attention as the production of eggs with broken, cracked or soft shells incurs significant economic losses. Eggshells of laying hens are highly organized mineral structures built of spherical calcite crystals deposited on the outer surface of the protein membrane, around egg white. The crystals of calcium carbonate are formed by crystallization from secreted supersaturated solution in the distal part of the oviducts. It takes about 20 hours for the formation of an eggshell, which indicates a large demand for a constant supply of the necessary amounts of calcium (Vitorovic et al., 2004).

During the laying period, the first challenge is to adjust the energy and protein requirements to optimize egg output and to carefully control body weight. The crude protein concentration and amino acids in the layer diet are also important, methionine being the main limiting amino acid. The provision of insufficient dietary calcium during the rearing or laying period has an adverse effect on both eggshell quality and bone strength (Bain et al., 2016). The laying hens requirement for dietary calcium within the diet for different ages is in the range from 0.9 to 1.2% during the growth period of the pullet, increasing to 2 to 2.5% just prior to the onset of lay and 3.5 to 4.5% once a lay is established (Bouvarel *et al.*, 2011).

Shell formation takes place mainly during the night when consumption is very low. Therefore, it is recommended to replace a portion of a fine pulverized calcium source in the diet with a larger particle size limestone, which takes more time to dissolve in the digestive tract, and thus presents calcium available overnight (Pavlovski et al., 2003). It is also recommended to implement the so-called night diet, when hens are stimulated to feed intake by periodic lighting.

Having all the above mentioned in mind, the aim of this study was to determine the influence of two nutritive supplements, as additional calcium sources administered in the drinking water on the quality of the eggshell.

## MATERIALS AND METHODS

The experiment was conducted on 34200 Lohmann Brown laying hens, 78 weeks of age. All of them were fed with a mixture of standard ingredient structure as shown in the Table 1, and the chemical composition shown in the Table 2.

Table 1. Ingredient composition of feed mixture

Ingredients	Composition [%]
Maize	56.00
Sunflower meal	12.50
Soybean cake	8.75
Soybean oil	0.75
Cereal meal	7.50
Mineral chalk	3.75
Grit 2-4 mm	6.25
Yeast	2.50
Premix	2.00
TOTAL	100.00

Premix composition [%] was the following: Cereal flour 46.393, Sodium bicarbonate 12.500, Monocalcium phosphate 12.386, Sodium chloride 9.508, Lysine 7.962, Methionine 4.968, Hepatron 95%/Choline 1.579, Mangan sulphate 1.094, Iron sulphate 0.968, Zinc sulphate 0.877, Axtra XB 201 laying hens 0.500, Vit. E 50 S 0.400, Copper sulphate 0.160, Axtra phytase TPT 0.150, Vit. B3 Niacin 0.147, Microgran Se 1% 0.125, Vit. B5 98% Pantothenic acid 0.061, Microgran I 10% 0.050, Vit. A 1.000.000 IU/g 0.050, Vit. K 51% 0.029, Vit. B2 80% 0.025, Vit. D3 500.000 IU/g 0.025, Vit. B6 99% 0.020, Vit. Biotin 2% 0.012, Vit. B12 1% 0.008 and Vit. folic acid 80 0.002.

Table 2. Chemical composition of feed mixture

Component	Units	Value	Component	Units	Value
MEpoultry	kcal	2734.665	Vit E	IE/g	0.040
MEpoultry	MJ	11.437	Vit K	mg/kg	3.000
Crude protein	g/kg	144.759	Vit B1	mg/kg	3.250
Crude fat	g/kg	35.617	Vit B2	mg/kg	5.000
Crude ash	g/kg	125.071	Vit B6	mg/kg	5.000
Crude fiber	g/kg	46.961	Vit B12	µg/kg	15.000
Calcium	g/kg	40.310	Niacine	mg/kg	40.000
Phosphor	g/kg	5.905	FolicAcid	mg/kg	0.750
AvPhosphrPoultr	g/kg	3.300	Biotin	µg/kg	50.000
Sodium	g/kg	1.700	Betaine	mg/kg	300.000
Cl	g/kg	1.872	Fe	mg/kg	60.000
Lysine	g/kg	7.500	Cu	mg/kg	8.000
Methionine	g/kg	3.748	Zn	mg/kg	60.000
Methionine+Cyst	g/kg	6.400	Mn	mg/kg	70.000
Threonine	g/kg	5.484	I	mg/kg	1.000
Tryptophane	g/kg	1.545	Se	mg/kg	0.250
Vit A	IE/g	10.000	Ca-pan- thotenate	mg/kg	12.000
Vit D3	IE/g	2.500			

Cage rearing facility consisted of three batteries, each containing 184 cages. The layers were thus divided into 3 experimental groups consisting of 11,400 individuals per each (R, C and L), which were of uniform performance and egg production characteristics. Every group had its own dispenser of drinking water. During 8 hours a day an experimental group R was given a product which contained calcium 5 g/l and phosphorus 144 g/l, in the dosage of 1 l per 1000 l of drinking water. At the same time experimental group L received another product containing calcium 75 g/l and vitamin D3 300 000 IU/l, in a dosage of 1 l per 1000 l of drinking water. For the control group C only pure water was provided. As different additives were used, it was not the goal to make a comparison between them, but to gain, in practical farm conditions, a quick insight into their impact in relation to the control group.

The experiment lasted for 15 days. During that period, there were five samplings of eggs, always within the same central part of the hall. The same number of eggs (30 eggs of A and 30 eggs of SS class) were taken from each group separately. Out of every 30 eggs, 14 were selected for weight measurements, with 2 lightest and 2 heaviest eggs discarded from statistics. The remaining 10 eggs were measured for the eggshell thickness using a micrometer after rinsing the shell in water to remove any adhering albumen. Later on, in order to determine the eggshell weight, drying at 105°C for two hours was performed with the shell membrane intact. This was followed by weighing on an analytical scale. The percentage of eggshell breakage was also recorded as a percentage of the cracked eggs in relation to the number of whole eggs.

Statistical analysis was done using Microsoft Excel 2007, while the differences in measurements between the groups were tested by paired two-tailed t-test and ANOVA Two-Factor.

## **RESULTS AND DISCUSSION**

The obtained results are given for each parameter separately as follows: The number of produced and cracked eggs (Table 3), the percentage of eggshell breakage (Table 4), Egg weight (Table 5), Eggshell weight (Table 6) and Eggshell thickness (Table 7).

Table 3. Number of eggs / Number of cracked eggs

Group	1 <sup>st</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day
R	9964/ 277	10386/ 320	10185/ 279	10114/ 290	11180/ 356
L	9916 / 272	10138/ 240	9971 / 286	9919 / 281	10921/ 320
C	9927 / 272	10132 / 275	9886 / 310	9780 / 305	10521/ 343

Table 4. Percentage of eggshell breakage [%]

Group	1 <sup>st</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day
R	2.78	3.08	2.74	2.87	3.18
C	2.74	2.36	2.86	2.83	2.93
L	2.74	2.71	3.13	3.12	3.26

Table 5. Average egg weight [g]

Group	Egg class	1 <sup>st</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day
R	A	62.11±0.87	62.96±1.15	61.70±1.43	62.81±1.02	63.14±0.91
	SS	71.78±0.75	72.23±0.88	72.49±0.75	72.96±0.75	73.08±0.88
C	A	62.17±1.14	62.87±1.28	61.57±0.93	63.38±1.09	63.59±1.01
	SS	71.90±0.74	72.32±0.87	72.64±0.68	72.29±0.95	73.29±0.73
L	A	62.32±1.29	62.04±1.16	61.42±2.18	62.95±0.86	62.63±1.51
	SS	71.92±0.97	72.62±0.73	73.59±1.19	73.06±1.02	72.08±0.45

Table 6. Average eggshell weight [g]

Group	Egg class	1 <sup>st</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day
R	A	6.523±0.712	6.919±0.787	6.586±0.580	6.618±0.454	6.705±0.633
	SS	7.124±0.735	7.151±0.672	7.204±0.953	7.501±0.557	7.098±0.730
C	A	6.395±0.354	6.939±0.606	6.535±0.344	6.911±0.549	6.731±0.512
	SS	7.375±0.461	7.589±0.458	7.465±0.654	6.992±0.559	7.186±0.675
L	A	6.491±0.785	7.002±0.559	7.066±0.569	6.790±0.865	6.623±0.585
	SS	7.150±0.670	7.671±0.615	7.770±0.700	7.232±0.635	7.332±0.557

Table 7. Average eggshell thickness [mm]

Group	Egg class	1 <sup>st</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day
R	A	0.351±0.037	0.362±0.038	0.351±0.049	0.339±0.023	0.361±0.032
	SS	0.353±0.039	0.356±0.039	0.333±0.037	0.342±0.020	0.355±0.033
C	A	0.345±0.030	0.349±0.042	0.329±0.023	0.356±0.025	0.355±0.023
	SS	0.351±0.028	0.364±0.033	0.331±0.040	0.324±0.021	0.358±0.030
L	A	0.349±0.028	0.365±0.026	0.368±0.028	0.356±0.034	0.356±0.014
	SS	0.350±0.026	0.368±0.030	0.348±0.021	0.364±0.025	0.354±0.017

These tables show that there were no statistically significant differences in eggshell quality parameters between the experimental groups. The only parameter that differed significantly comparing to the control group ( $< 0.01$ ) was the number of eggs in the group R, which was given product containing calcium and phosphorus. Dissimilarity of all other results had no statistical importance.

Some authors, like Tunc and Cufadar (2015), who investigated influence of dietary large calcium sources (limestone, oyster shell and egg shell) on performance and eggshell quality parameters in laying hens observed no effect. However, the diet used in their experiment, containing at least 50% large calcium sources, had positive effect on mineral contents of tibia. Also, in earlier research of Keshavarz and Nakajima (1993) no benefit of adding more calcium to limit the age deterioration in shell quality was determined, as well as no influence of such dietary treatments on egg production. This could be due to some other factors like breed and age of laying hens, inheritance and the environmental conditions, which could be dominant. Increasing the dietary level of Ca without beneficial effects on shell quality indicate that the National Research Council (NRC, 1994) estimation of calcium (Ca) requirement of 3.25 g per hen per day is adequate for optimum shell formation.

On the other hand, experiment performed by Zhang et al. (2017), which lasted for 10 weeks, showed that aged non-molted laying hens (77 weeks) or older molted second cycle layers (94 weeks) require lower calcium solubility and higher calcium intake compared to relative younger laying hens (36 weeks old) to maximize shell quality and bone status. The amounts 3.94 - 4.89 g of Ca intake for a hen per day from calcium carbonate source with a solubility range of 30.1-39.8% was recommended for older layers. Therefore, according to these authors, the NRC recommendation might not be sufficient to support the performance variables such as egg shell quality and bone status in older non-molted laying hens, or older molted second cycle laying hens. Pavlovski

et al. (2003) and Vitorovic et al. (2004) similarly suggested usage of limestone of larger particle size to improve eggshell quality of laying hens, while Wang et al. (2013) had the same conclusion for ducks.

Sharma et al. (2009) used liquid additional supplement source of calcium and phosphorus in their experiment and showed that overall egg and shell quality was improved in correlation with the activity of herbal constituents of the products owing to mineralization properties. Jadhav et al. (2010) also proved that supplementation of the same liquid calcium and phosphorus source was efficacious in enhancing bioavailability of these minerals, thereby improving overall performance and bone mineralization in broilers.

During two weeks of our experiment, additional calcium from liquid source given through drinking water, especially supplement with calcium and vitamin D3 in group L, had no significant impact on the examined parameters. It possibly means that the maximal exploitation of hens, in these circumstances, was achieved through adequate nutrition. In the used complete feed all nutrients were balanced and aligned with the needs of Lohmann Brown breed, while feed calcium was at the level of 3.95%. On this issue McDowell (2017) emphasized that the usage of liquid supplements to provide minerals might not provide enough calcium due to the solubility problems. Also, the duration of the experiment of only two weeks is a possible limiting factor.

Amino acids, in particular methionine and lysine (Bain et al., 2016), as well as fatty polyunsaturated linoleic acid are also important for adequate egg production. Certain improvement in the trial was observed in the treatment with the product containing calcium and phosphorus, which indicates the significance of both minerals and demonstrates the importance of balancing Ca-P ratio in the diet of laying hens (Sefer and Sinovec, 2008). The establishment of Ca and P requirements of commercial layers is a continuous challenge for poultry nutritionists and egg producers as the needs for these two minerals seem to constantly change (Pelicia et al., 2009).

Two-week experiment seems to be a short time to gain a quick insight into the impact of the applied supplements within the farm conditions and in limited material possibilities, compared to the five-week trial carried out by Jadhav et al. (2010). In order to get a clearer picture and deeper understanding of obtained results, the continuity of investigations by extending the experiment, or by examining some other sources and diet regimens of laying hens are required. Also, as no significant differences in effects of two additives of different composition were found, this research limitation has no influence on final conclusions, but affirms the need to engage these complex facts in further investigations as well.

## CONCLUSION

Based on the obtained results and literature data, it could be concluded that the amount and source of calcium in the diet of laying hens is a very complex and still not fully solved issue. Many factors influence the quality of eggshell. Taking into account significant economic losses incurred by the production of eggs with broken, cracked or soft shells, as well as demand to increase food production for 60% by the middle of this century, it is obvious that all research efforts for improvement in breeding and production of laying hens are necessary and important. The extension of the research is required and the topic of this paper should also be addressed.

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