POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2007, Vol. 57, No. 4(A), pp. 107-111

EFFECT OF CALCIUM PEROXIDE ON THE PERFORMANCE, EGG QUALITY AS WELL AS CALCIUM AND PHOSPHORUS CONCENTRATIONS IN THE BLOOD SERUM OF LAYING HENS

Zbigniew Dobrzański[1], Sebastian Opaliński[1], Mariusz Korczyński[1], Barbara Walawska[2], Tadeusz Trziszka[3], Magdalena Trafankowska[1]

[1]Department of Animal Hygiene and Environment, Wroclaw University of Environmental and Life Sciences, Wrocław; [2]Institute of Inorganic Chemistry in Gliwice; [3]Department of Animal Products Technology and Quality Management, Wroclaw University of Environmental and Life Sciences, Wroclaw

Keywords: calcium peroxide, laying hens, performance, egg, blood serum

The examination was made in the experimental room where 36 laying hens were kept in battery system. The hens were divided into three groups (12 birds in each group) – one control group (C) and two experimental groups E-1 and E-2. The birds from the control group were fed with the all-mash prepared according to standard recipe; the content of calcium was 3.0%. The feed for the experimental groups contained also 3% of calcium but 0.5% (E-1) and 1.0% (E-2) of Ca was from CaO_2 (51.8% of Ca, 16.7% of active oxygen, pH-value in 1% solution – 12.5). The experiment lasted 56 days. Total calcium concentration in blood serum samples and chosen qualitative parameters of eggs were analysed.

It was found that eggshells thickness from laying hens of group E-2 was higher comparing with eggshells from control group and from group E-1 ($p \leq 0.01$). The eggshells from both experimental groups were more resistant (breaking strength) than eggshells from group C, especially the samples collected after 2 and 4 weeks of experiment ($p \leq 0.01$ and $p \leq 0.05$). The eggs from groups E-1 and E-2 had higher weight but that fact was not statistically confirmed. Calcium peroxide used in our experiment did not cause any discolouration of eggshells and had no influence on the colour of egg yolk. Calcium concentrations in blood serum were high (4.43 to 6.74 mmol/L) but not exceeded the values reported by other authors.

INTRODUCTION

An eggshell mainly consists of calcium carbonate (97%) so the whole egg contains 2.00-2.69 g of pure calcium. In order to form appropriately resistant eggshell, hen organism should be provided with optimum calcium amount in a feed ration. Daily calcium demand for one eggshell is about 2.3 g [Trziszka, 2000]. The calcification process of egg in the shell gland is proceeding usually thorough the night, when intake of feed is very low, and takes about 21 hours [Nys *et al.,* 2001].

Therefore, physical form of calcium additives used in laying hens feeding is of a considerable importance. Investigations made by many authors [Jamroz *et al.*, 2000; Koreleski *et al*., 2005; Narvaez-Solarte *et al*., 2006] proved that optimal granulation form of calcium supplements are pellets of 2-4 mm diameter. The mentioned parameter enables longer Ca release in a gizzard both during the day and at night. Due to this fact hen organism utilizes calcium deposits in bones to a lesser degree.

Apart from appropriate calcium quantity in feed ration, quite important role in building eggshell is played by other factors like feed pH, magnesium, zinc, vitamin D_3 amount, as well as proper calcium-phosphorus ratio, which should equal 12:1 [Boorman & Gunarantne, 2001; Jamroz *et al*., 2000]. It was also found that the level of calcium absorption from laying hen's digestive tract decreases as the birds get older. Especially in the last period of intensive egg laying, absorption of this chemical element decreases to the level below 30 or even 25%, therefore, calcium contribution to feed matter should be increased [Narvaez-Solarte *et al*., 2006; Kermanashahi & Hadavi, 2006]. Prolonged low calcium level in feed mixture is resulted in diminished laying, egg weight and eggshell breaking strength. The eggshells which are fragile can cause very big losses (high number of cracked eggs), especially in the poultry farms, where birds are kept in battery system.

In practice, the following additives are used as Ca source for laying hens: milled oyster shells, milled eggshells, limestone or phosphates [Ajakaiye *et al*., 1997; Abd-Elrazig & Elzubeir, 1998; Jamroz *et al*., 2000]. Alternative source of feed calcium can be calcium peroxide (CaO_2). This oxide contains more than 50% of Ca and also active oxygen [Walawska & Gluzińska, 2006b].

The aim of the investigation was to determine the influence of calcium peroxide, as calcium source in feed mixture, on eggshells and egg yolk quality, as well as on calcium and phosphorus content in blood serum of laying hens as the criterion of its availability.

MATERIALS AND METHODS

The examination was made in the experimental room (precisely controlled conditions) where 36 laying hens (Lohmann

Author's address for correspondence: Zbigniew Dobrzański, Department of Animal Hygiene and Environment, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 38C, 51-630 Wrocław, Poland; tel.: (48 71) 320 58 66; e-mail: khz@ozi.ar.wroc.pl

Brown) in the first period of egg production, between 42 and 50 weeks of life, were kept in battery system. During the experiment, the standard lighting program was applied; feed and water were available *ad libitum*. The birds were divided into 3 groups (12 hens in each) – control group (C) and two experimental groups (E-1 and E-2). Birds were fed with the all-mash feed type J-297 (Tables 1 and 2), prepared according to standard recipe, the content of calcium was 3.0%. The feed for the experimental groups contained the same amount of calcium as the feed for the control group but 0.5% (E-1) and 1.0% (E-2) of Ca was supplemented by addition of CaO_2. The daily intake of feed for one laying hen was at the level of 130 g/day. The experiment lasted 56 days.

TABLE 1. Basic nutrients content in J-297 type feed mixture for laying hens.

Nutrients	Declared content
Dry matter (%)	89.6
Crude protein (%)	17.00
Crude fibre (%)	3.20
Crude ash (%)	10.00
Crude fat (%)	5.00
Methionine (%)	0.38
Metabolizable energy (kcal/kg)	2780
Ca content (%)	3.00
P content (%)	0.62

TABLE 2. The content of feed additives in 1 kg of the all-mash type J-297 feed supplemented with premix in the amount of 0.5%.

Feed additive	Unit	Content
Vitamin A	μ/kg	10 000
Vitamin D_3	μ/kg	2 500
Vitamin E	mg/kg	20.00
Vitamin K_3	mg/kg	1.52
Vitamin B_1	mg/kg	1.50
Vitamin B_2	mg/kg	5.00
Nicotinic acid	mg/kg	30.00
Panthothenic acid	mg/kg	10.00
Vitamin B_6	mg/kg	2.00
Vitamin B_{12}	mg/kg	15.00
Biotin	mg/kg	50.00
Folic acid	mg/kg	1.05
Ca	%	0.16
Fe	mg/kg	40.00
Mn	mg/kg	80.00
Zn	mg/kg	50.00
I	mg/kg	1.02
Co	mg/kg	0.50
Se	mg/kg	0.25
Cu	mg/kg	10.00
Antioxidant	mg/kg	15.00
Enzyme Natuphos	mg/kg	80.00

Calcium peroxide contained 16.7% of active oxygen, 51.8% of calcium and pH value of its 1% solution was 12.5. The investigated peroxide was prepared from pure reagents according to the method elaborated by the Institute of Inorganic Chemistry in Gliwice [Walawska & Gluzińska, 2006 a,b].

The following parameters like feed intake, number and weight of laid eggs were daily controlled. Eggs for examinations were collected four times (30 eggs out of each group), *i.e.* on 14th (I series), 28th (II), 42nd (III) and 56th (IV) day of the experiment.

During the laboratory investigation the following parameters were analysed: (1) weight of eggshells (g – with accuracy to 0.1 g); (2) the colour of egg yolk according to the La Roche scale (1-15), (3) thickness of eggshells (mm) on egg equator at two points with the use of the Wolpert – IP94 micrometer screw (accuracy to 1 μm); and (4) breaking strength of eggshells (N) with the use of the Zwick/Roell device.

In the second (I series), sixth (III) and eighth week (IV) of the experiment blood samples were taken from a wing vein of experimental hens (8 hens out of each group). The concentrations of total calcium and inorganic phosphorous in blood serum were analyzed by colorimetric method with the use of the biochemical analyzer Pentra 2000 (Horiba reagents).

The results were elaborated statistically (Statgraphics software ver. 5.1) by calculating the mean values and standard deviations. The assessment of the differences between values obtained for experimental groups in each series of the experiment were done (Duncan test).

RESULTS AND DISCUSSION

Production results

In all groups average level of laying was lower at the beginning of the experiment than the one obtained at the end and it ranged from 82.5 to 87.5%, which was caused by the fact that birds were entering their laying peak. Feed intake for one egg production did not statistically differ amongst the groups. Similarly, comparison of average egg weight within the whole experiment did not point to any effect of Ca supplementation from calcium peroxide. However, increase of egg weight in E-1 and E-2 groups (Table 3), especially in the final week of the experiment, could be observed, although that difference was not statistically confirmed. No cracked or low quality eggs were found in particular groups.

Obtained production results were typical for this laying hen's line [Świątkiewicz & Koreleski, 2007] so the calcium peroxide supplementation had no effect on laying rate, feed intake nor egg weight.

Egg quality parameters

Evaluating parameters of eggshells obtained from groups E-1 and E-2 it was found that the mean value of eggshell thickness increased, especially in 4th and 6th week of experimental period (II and III series), in comparison to those from control group (Table 4). In comparison to the eggs from control group the highest increase ($p \leq 0.01$) was obtained for the eggs of E-2 group (II and III series) and for the eggs of E-1 group (III series) the increase was slightly lower ($p \leq 0.05$). In the 8th week (IV series) the mean value of eggshell thickness became even. That

TABLE 3. Egg performance determined within 8-week experiment.

Group	Laying rate(%)		Feed intake per		Number of eggs laid (pcs)	Weight of eggs laid (kg)	Egg weight (g)
	initial	end	egg (g)	hen/day (g)			
C	82.5	94.0	139.0	128.7	598	32.54	54.4
E-1	87.5	96.9	132.8	131.1	611	35.50	58.1
E-2	82.8	91.8	142.2	127.5	592	33.23	56.2

TABLE 4. Eggshell thickness (mm).

Group		I series	II series	III series	IV series	Total ($\overline{x}$ ± SD)
C	$\overline{x}$	0.418	0.419^{A}	0.418^{Aa}	0.420	0.42 ± 0.019^{A}
	SD	0.011	0.02	0.025	0.016	
E-1	$\overline{x}$	0.421	0.438	0.439^{b}	0.435	0.43 ± 0.02^{B}
	SD	0.011	0.011	0.019	0.025	
E-2	$\overline{x}$	$0.425^{**/*}$	0.453^{B**}	0.450^{B*}	0.418^{**}	0.44 ± 0.02^{B}
	SD	0.02	0.018	0.018	0.009	

**= p<0.01 and *= p<0.05 – significance of differences in each group between following weeks; A, B= p<0.01 and a, b= p<0.05 – significance of differences between the experimental groups in following weeks

TABLE 5. The breaking strength of an eggshell (N).

Group		I series	II series	III series	IV series	Total ($\overline{x}$ ± SD)
C	$\overline{x}$	41.17^{a}	41.86^{A}	41.15^{a}	41.17^{a}	41.34 ± 2.16^{A}
	SD	1.87	2.97	2.41	1.87	
E-1	$\overline{x}$	43.31	45.85^{B}	42.73	43.31	43.80 ± 2.83^{B}
	SD	2.10	3.78	2.58	2.10	
E-2	$\overline{x}$	45.76^{b}	42.90^{B}	44.97^{b}	45.76^{b}	44.74 ± 4.37^{B}
	SD	2.36	6.53	5.00	2.36	

A, B= p<0.01 and a, b= p<0.05 – significance of differences between the experimental groups in following weeks

alteration was caused by decrease of average eggshell thickness in E-2 group in comparison to earlier collection terms (p≤0.01). Świątkiewicz and Koreleski [2007] obtained lower values of eggshell thickness (mean value 0.373 mm) for the same line of laying hens but the birds were younger (26– 28 week of life).

Calcium supplementation from CaO_2 resulted in the increase of eggshell breaking strength (Table 5). It was most evident in experimental groups after 4 weeks of experiment in comparison to C group (p≤0.01). In E-2 group the eggshells from the eggs collected during I and III series were also more resistant (p≤0.05) comparing with C group eggs and furthermore that tendency lasted until the end of experiment (IV series). In the case of E-1 laying hens, eggshells collected in the last experimental series had lower average breaking strength as compared to the E-2 group, but not differing statistically from mean value of that parameter determined for C group eggshells. Obtained breaking strength parameters were similar to the results reported by other authors [Świątkiewicz & Korelski, 2007], but they were more satisfactory than those of Hy Line hens [Korelski & Świątkiewicz, 2005].

The improvement of eggshell quality can be obtained by calcium addition before turning the light off, according to lighting program [Kermanshabi & Hadavi, 2006]. Vitamins of D group, as well as some macroelements are also of considerable meaning [Jamroz, 2004]. According to Chowdhury & Smith [2002], eggshell quality improved when hens were fed 3.5% calcium diet in combination with 0.10% putrescine (1.4--diaminobutane). Also supplementation of microbial phytase at a level of 300 U per kg diet of laying hens can improve egg production, decrease broken and soft egg production rate, and P excretion [Lim *et al.,* 2003]. On the other hand, the addition of 2-3% of phosphogipsum to feed mixture resulted in decreasing eggshell breaking strength values [Górecki *et al.*, 2006].

Introduction of calcium peroxide to laying hens feed mixture did not result in any significant differences in egg yolk colour. According to La Roche scale, the mean value obtained for C group egg yolk was 13.0 and for E-1 and E-2 egg yolks was 12.8 and 11.5 respectively, which can suggest certain effect of CaO_2 supplementation on egg yolk colour, however,

TABLE 6. Concentrations of total calcium and inorganic phosphorus in laying hens blood serum (mmol/L).

Group		I series		III series		IV series	
		Ca	P	Ca	P	Ca	P
C	$\overline{x}$	4.48^{b}	1.73^{B}	5.00	1.81	6.27	2.02
	SD	0.58	0.33	0.82	0.35	1.08	0.26
E1	$\overline{x}$	5.47^{a}	2.56^{A}	5.71	2.21	6.74	2.51
	SD	0.92	0.55	0.81	0.14	0.84	0.42
E2	$\overline{x}$	4.49	1.72^{B}	4.95	2.06	5.82	2.12
	SD	1.13	0.39	1.02	0.47	1.01	0.47

A, B= $p<0.01$ and a, b= $p<0.05$ – significance of differences between the experimental groups in following weeks

those differences were not statistically significant. Another investigation proved discolouring of egg yolk when humine chalk was applied [Dobrzański *et al*., 2000] and after the introduction of humine-mineral or humine-fatty preparation alike [Rudnicka & Dobrzański, 2000]. The discolouration of eggshell and yolk can be cause by some vegetable feed additives which are used in poultry feeding *i.e.* guar by-product [Gutierrez *et al.*, 2007].

Calcium and phosphorus in blood serum

Determined levels of total calcium and inorganic phosphorus (Table 6) in the examined hens blood serum samples, collected in early morning hours, were considerably high, yet meeting the range of the values reported by other authors [Eren *et al*., 2004; Jamroz *et al*., 2000]. That fact could serve as a proof of appropriate availability of calcium at night in the examined laying hens. However, it is difficult to explain statistically significant differences between the concentration of P in group E-1 and other groups and the concentration of Ca between group E-1 and C group for blood samples collected during the first series.

Calcium supplementation from CaO_2 did not cause any disorders of calcium-phosphorus ratio in blood of experimental hens. Phosphorus fraction in ratio to calcium in blood serum oscillated within the range 1:2 to 1:3. The content of Ca and P in serum depends on many factors, their concentration may be decreased by high environment temperature and addition of vegetable oil to hen diet improved only serum P [Usayran *et al.,* 2001].

CONCLUSIONS

The supplementation of calcium peroxide to the feed for laying hens is a promising alternative. The examined eggs collected from experimental groups during research were characterized by improvement of eggshell strength without negative influence on the egg laying rate as well as on the eggs mass. However, obtained initial results of the study are relatively difficult to be interpreted since laying hens were in the period of high egg laying. Nevertheless, they point to purposefulness of further investigation, especially taking into account the final stage of egg laying when natural, physiological effect of decreasing eggshell strength and worsening of egg quality take place.

REFERENCES

1. Abd-Elrazig S., Elzubeir E.A., Effects of feeding pearl millet on laying hen performance and egg quality. Anim. Feed Sci. Technol., 1998, 76, 89-94.
2. Ajakaiye A., Atteh J.O., Leeson S., Effects of calcium source, particle size and time on in-vitro calcium solubility of some indigenous Nigerian mineral ingredients for pultry diets. Anim. Feed Sci. Technol., 1997, 65, 239-298.
3. Boorman K.N., Gunaratne S.P., Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. Brit. Poult. Sci. 2001, 42, 81-91.
4. Chowdhury S.R., Smith T.K., Dietary interaction of 1,4-diaminobutane (putrescine) and calcium on eggshell quality and performance in laying hens. Poult. Sci., 2002, 81 (1), 84-91.
5. Dobrzański Z., Rudnicka A., Trziszka T., Effect of dietary raw limestone on the quality and chemical composition of hens eggs. Zesz. Nauk. AR Wrocław, ser. Zoot., 2000, 47, 35-43 (in Polish; English abstract).
6. Eren M., Uyanik F., Kucukersan S., The influence of dietary boron suplementation on egg quality and serum calcium, inorganic phosphorus, magnesium level and alkalain phosphatase activity in laying hens. Res. Vet. Sci., 2004, 76, 203-210.
7. Górecki H., Chojnacka K., Dobrzański Z., Kołacz R., Górecka H., Trziszka T., The effect of phosphogypsum as the mineral feed additive on fluorine content in eggs and tissues of laying hens. Anim. Feed Sci. Technol., 2006, 128, 84–95.
8. Gutierrez O., Zhang C., Cartwright A.L., Carey J.B., Bailey C.A., Use of guar by-products in high-production laying hen diets. Poult. Sci., 2007, 86, 1115-1120.
9. Jamroz D., Skorupińska J., Laskowska B., Use of different calcium sources for laying hens. Zesz. Nauk. AR Wrocław, ser. Zoot., 2000, 46, 69-86 (in Polish; English abstract).
10. Jamroz D., Wapń. 2004, *in:* Żywienie zwierząt i paszoznawstwo, Fizjologiczne i biochemiczne podstawy żywienia zwierząt, t.1 (ed. Dorota Jamroz). Wyd. Nauk. PWN, Warszawa, pp. 63-67 (in Polish).
11. Kermanshahi H., Hadavi A., Effect of added extra calcium carbonate into the diets, one hour before starting dark period on performance and egg quality of laying hens. Int. J. Poult. Sci., 2006, 5 (10), 946-948.
12. Koreleski J., Świątkiewicz S., Efficacy of different levels of a cholecalciferol 25-0H derivative in diets with two limestone forms in laying hen nutrition. J. Anim. Feed Sci., 2005, 14, 305-315.

13. Lim H.S., Namkung H., Paik I.K., Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. Poult. Sci., 2003, 82, 92-99.
14. Narváez-Solarte W., Rostagno H.S., Soares P.S., Uribe-Velasquez F.S., Silva M.A., Nutritional requirement of calcium in white laying hens from 46 to 62 week of age. Int. J. Poult. Sci., 2006, 5, 181-184.
15. Nys Y., Gautron J., McKee M.D., Garcia-Ruiz J.M., Hincke M.T., Biochemical and functional characterisation of eggshell matrix proteins in hens. World´s Poult. Sci., 2001, 57, 401--413.
16. Rudnicka A., Dobrzański Z., The effect of humic and fatty dietary preparations on productivity and egg quality of molted Lohmann Brown hens. 2000, *in*: Proceedings Xth Intern. Congr. Anim. Hyg., vol. 1, Maastricht, The Netherlands, pp. 247-251.
17. Świątkiewicz S., Koreleski J., Quality of egg shells and bones in laying hens fed a diet containing distillers dried grains with solubles. Medycyna Wet., 2007, 63, 99-103 (in Polish; English abstract).
18. Trziszka T., Fizjologia formowania się jaja oraz wady jaj. 2000, *in*: Jajczarstwo, nauka, technologia, praktyka (ed. Tadeusz Trziszka). Wyd. AR we Wrocławiu, p. 138 (in Polish).
19. Usayran N, Farran M.T, Awadallah H.H, Al-Hawi I.R, Asmar R.J, Ashkarian V.M., Effects of added dietary fat and phosphorus on the performance and egg quality of laying hens subjected to a constant high environmental temperature. Poultry Sci., 2001, 80, 1695-1701.
20. Walawska B., Gluzińska J., Effect of H_2O_2 concentration of the efficiency of calcium peroxide preparation. Chem. Agric., 2006a, 7, 17-21.
21. Walawska B., Gluzińska J., Calcium peroxide – properties and application for protection of the environment. Chem. Agric., 2006b, 7, 804-807.

WPŁYW NADTLENKU WAPNIA NA PARAMETRY PRODUKCYJNE, JAKOŚCIOWE JAJ ORAZ ZAWARTOŚĆ WAPNIA I FOSFORU W KRWI KUR NIEŚNYCH

Zbigniew Dobrzański[1], Sebastian Opaliński[1], Mariusz Korczyński[1], Barbara Walawska[2], Tadeusz Trziszka[3], Magdalena Trafankowska[1]

[1] Zakład Higieny Zwierząt i Środowiska, Uniwersytet Przyrodniczy we Wrocławiu; [2]Instytut Chemii Nieorganicznej w Gliwicach; [3] Katedra Technologii Surowców Zwierzęcych i Zarządzania Jakością, Uniwersytet Przyrodniczy we Wrocławiu

Celem pracy było określenie wpływu nadtlenku wapnia (CaO_2), jako źródła wapnia w paszy, na wyniki produkcyjne, wybrane parametry jakościowe jaj oraz zawartość wapnia całkowitego w surowicy krwi u niosek, jako kryterium jego bioprzyswajalności. Badania przeprowadzono w wiwarium na materiale 36 młodych kur nieśnych linii Lohmann Brown, utrzymywanych w systemie bateryjnym przez okres 8 tygodni. Stosowano standardowy program świetlny oraz zapewniono stały dostęp do paszy i wody. Ptaki podzielono na 3 grupy (po 12 niosek w grupie) – grupę kontrolną (K) oraz dwie grupy doświadczalne (D-1- i D-2). Ptaki otrzymywały pełnoporcjową mieszankę paszową typu J-297 o zawartości wapnia 3%. Z paszy dla grup D-1 i D-2 wycofano odpowiednio 0,5% i 1,0% czystego wapnia i uzupełniono poprzez dodatek wapnia z CaO_2. Kontrolowano wskaźniki produkcyjne (nieśność, masa jaja, pobranie paszy), określono wybrane parametry jakościowe jaj. Jaja do badań pobrano w 4 seriach: w 14, 28, 42 i 56 dniu trwania doświadczenia. W surowicy krwi oznaczono poziom wapnia całkowitego oraz fosforu nieorganicznego.

Stwierdzono wzrost grubości skorupy jaja od kur z grupy D-2 w stosunku do grup kontrolnej i D-1 ($p\leq0,01$). W obu grupach doświadczalnych stwierdzono wzrost wytrzymałości skorup jaj w stosunku do jaj z grupy kontrolnej, szczególnie po 4 i 6 tygodniach podawania preparatu ($p\leq0,01$ i $p\leq0,05$). Jaja od kur otrzymujących nadtlenek wapnia charakteryzowały się wyższą masą, lecz nie zostało to potwierdzone statystycznie. Podawany nadtlenek wapnia nie powodował odbarwień żółtka jaja. Stężenie Ca i P w surowicy krwi kur było dość wysokie, lecz mieściło się w zakresie wartości fizjologicznych, co świadczy o prawidłowym zaopatrzeniu niosek w wapń.