Ann. Anim. Sci., Vol. 18, No. 1 (2018) 167–183 DOI: 10.1515/aoas-2017-0038

DE GRUYTER
OPEN

EFFECT OF SELECTED FEED ADDITIVES ON EGG PERFORMANCE AND EGGSHELL QUALITY IN LAYING HENS FED A DIET WITH STANDARD OR DECREASED CALCIUM CONTENT*

Sylwester Świątkiewicz[1♦], Anna Arczewska-Włosek[1], Józefa Krawczyk[1], Witold Szczurek[1], Michał Puchała[1], Damian Józefiak[2]

[1]National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[2]Department of Animal Nutrition and Feed Management, Poznań University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland
♦Corresponding author: sylwester.swiatkiewicz@izoo.krakow.pl

Abstract

The aim of the experiment with 240 ISA Brown hens fed the diets with standard or decreased Ca level was to evaluate the effect of selected feed additives on laying performance and eggshell quality. The hens were allocated to 10 treatments, each containing 12 cages (replicates) of 2 birds. A 2 × 5 experimental arrangement was used. From 26 to 70 wks of age, experimental diets containing 3.20 or 3.70% Ca were used. The diets were either not supplemented, or supplemented with sodium butyrate, probiotic bacteria, herb extracts blend or chitosan. The decreased dietary Ca reduced eggshell quality indices in older hens (43-69 wks) (P<0.05) without effect on performance indices. The addition of the probiotic, herb extracts, or chitosan increased the laying rate (P<0.05). In older hens, i.e. at 69 wk, chitosan increased eggshell thickness and breaking strength, while herb extracts increased eggshell thickness (P<0.05). There was no interaction between the experimental factors in performance and eggshell quality. The used feed additives had no influence on fatty acid profile of egg lipids, however diet supplementation with chitosan decreased cholesterol concentration in egg yolk lipids (P<0.05). It can be concluded that such feed additives as probiotic, herb extracts, or chitosan may positively affect performance and eggshell quality, irrespective of Ca dietary level.

Key words: laying hens, calcium, feed additives, eggshell quality, yolk fatty acid profile

Since cracked or broken eggshells account for 80–90% of routinely downgraded eggs (Mabe et al., 2003), the quality of eggshells, i.e. their resistance to breakage, is an important concern in the commercial poultry industry, influencing the eco-

*This work was supported from the National Research Institute of Animal Production statutory activity (Research Project No. 05-008.1; Balice, Poland).

nomic profitability of egg production. Roland (1988) reported that eggs with damaged shells can account for 6–10% of all produced eggs, which leads to significant economic losses at different stages of the egg production process. The eggshell quality is also important as regards the egg safety for human consumption, as lack of defects and a high eggshell resistance to breaking is necessary for protection against penetration by pathogenic bacteria into the eggs. One of the main issues concerning the quality of the eggshell is its decrease with the age of the layers, which can be attributed to the increase in egg weight without an increase in the amount of calcium carbonate deposited in the shells (Al-Batshan et al., 1994) and disorders in vitamin D_3 metabolism (Abe et al., 1982). For this reason, the amount of cracked eggs at the end of the laying period may even be higher than 20% (Nys, 2001). Therefore, effective methods to improve eggshell quality are of vital importance for the commercial egg industry.

Different nutritional strategies are considered to enhance the quality of eggshell in high-producing laying hens, however most of the studies have been focused on macrominerals and vitamin D_3 (Nys, 1999), and the crucial importance of dietary Ca, P and vitamin D_3 levels and sources for eggshell quality is well known. For instance, supplying the hen with an optimal Ca intake is the most critical nutritional factor in order to ensure the proper calcification of the eggshell, but increasing the dietary level of Ca above 3.6–3.9% usually has no beneficial effect on eggshell quality (Keshavarz, 2003; Pastore et al., 2012; Jiang, 2013). In our recent study (Swiatkiewicz et al., 2015 b), no differences in the eggshell quality parameters were found when the laying hens were fed a diet containing 3.20, 3.70 and 4.20% of Ca. However, the results of some experiments have shown that the use of a particulate limestone as a source of Ca for laying hens can be more efficient in terms of eggshell quality (Koreleski and Swiatkiewicz, 2004; Lichovnikova, 2007).

The findings of several experiments demonstrated that chosen feed additives may improve eggshell quality and that this influence may be attributed mainly to enhancing the availability of Ca and other minerals (Swiatkiewicz and Arczewska-Wlosek, 2012; Swiatkiewicz et al., 2015 a). The results of the studies on the efficacy of different feed additives in the nutrition of layers are not consistent, however some findings suggest that eggshell quality may be improved by diet supplementation with pre- and probiotics, organic acids, and herb extracts (Çabuk et al., 2006; Sengor et al., 2007; Swiatkiewicz et al., 2010; Abdelqader et al., 2013 a; Abdelqader et al., 2013 b; Lokaewmanee et al., 2014; Sobczak and Kozlowski, 2015; Olgun, 2016). Therefore, the goal of this study was to determine the effect of adding selected feed additives to the diet with standard or decreased level of calcium on egg performance and eggshell quality in high producing laying hens.

Material and methods

Birds and experimental diets

A total of 240, 18-wk-old ISA Brown hens were obtained from a commercial source and placed in cages, on a wire-mesh floor, and under controlled climate conditions, in the poultry house of the Experimental Station of the National Research Institute of Animal Production. The cage dimensions were 30 cm × 120 cm × 50 cm, equating to 3600 cm^2 total floor space. During the pre-experimental period, i.e. from 18 to 25 wks of age, a commercial diet (170 g/kg crude protein, 11.6 MJ/kg AME_N, 37.0 g/kg calcium and 3.8 g/kg available phosphorus) was offered *ad libitum*.

Table 1. Composition and nutrient content of experimental diets, g/kg air dry matter

Item	Reduced dietary level of Ca	Standard dietary level of Ca
Corn	417.1	423.1
Wheat	240.0	210.0
Soybean meal	230.0	236.0
Rapeseed oil	13.0	19.0
Limestone	78.0	90.0
Monocalcium phosphate	12.5	12.5
NaCl	3.0	3.0
DL-Methionine	1.4	1.4
Vitamin-mineral premix[1]	5.0	5.0
Calculated composition:		
metabolisable energy (MJ/kg[2])	11.60	11.60
crude protein	170.0	170.0
Lys	8.35	8.35
Met	4.10	4.10
Ca	32.0	37.0
total P	6.15	6.15
available P	3.90	3.90

[1]The premix provided per 1 kg of diet: vitamin A, 10,000; vitamin D_3, 3,000 IU; vitamin E, 50 IU; vitamin K_3, 2 mg; vitamin B_1, 1; vitamin B_2, 4 mg; vitamin B_6, 1.5; vitamin B_{12}, 0.01 mg; Ca-pantothenate, 8 mg; niacin, 25 mg; folic acid, 0.5 mg; choline chloride, 250 mg; manganese, 100 mg; zinc, 50 mg; iron, 50 mg; copper, 8 mg; iodine, 0.8 mg; selenium, 0.2 mg; cobalt, 0.2 mg.

[2]Calculated according to European Table (Janssen, 1989) as a sum of the ME content of components.

At wk 26, the hens were randomly assigned to one of 10 treatments, each comprising 12 replicates (cages with 2 hens in each) and fed experimental diets until wk 70. During the experiment, the hens had free access to feed and water, and were exposed to a 14 L:10 D lighting schedule, with a light intensity of 10 lux. The composition of the experimental cereal-soybean diets is given in Table 1. A 2 × 5 factorial arrangement was used. The experimental diets contained two dietary levels of Ca (reduced – 3.20% or standard – 3.70%). The diets were supplemented with five experimental additives: none, sodium butyrate (700 mg/kg, GUSTOR XXI B 70, NOREL S.A., Spain), probiotic bacteria (150 mg/kg, PROTEXIN commercial preparation containing in 1 g: *Lactobacillus plantarum* – 1.26 × 10^7 cfu, *Lactobacillus bulgaricus* – 2.06 × 10^7; *Lactobacillus acidophilus* – 2.06 × 10^7; *Lactobacillus rhamnosus* – 2.06 × 10^7; *Bifidobacterium bifidum* – 2.00 × 10^7; *Streptococcus thermophilus* – 4.10 × 10^7; *Enterococcus faecium* – 5.90 × 10^7; *Aspergillus oryzae* – 5.32 × 10^6), herb extracts blend (2000 mg/kg feed, 1 kg of blend provided: dry extract from *Echinacea purpurea*, 4000 mg; oleoresin *Salvia officinalis*, 27 800 mg; oleoresin *Thymus vulgaris*, 5000 mg; oil extract from *Rosmarinus officinalis* 2500 mg; oil from *Allium sativum*, 1670 mg; and oil from *Origanum vulgare*, 1000 mg; Intermag Sp. z o.o., Poland) or chitosan (100 mg/kg used as Chimet-pasz preparation, Gumitex Poli-Farm, Poland). The nutritional composition of the experimental diets was calculated based on the chemical composition of the raw feedstuffs and the metabolisable energy value on the basis of equations from European Tables (Janssen, 1989). The chemical composition of the feed materials was analysed using conventional methods (AOAC, 2000). Amino acids were determined in acid hydrolysates, after the initial peroxidation of sulphur amino acids, in a colour reaction with a ninhydrin reagent using a Beckman System Gold 126AA automatic analyser. Calcium content was determined using flame atomic absorption spectrophotometry and phosphorus content using the calorimetric method (AOAC, 2000).

Measurements

During the experiment, the feed intake, number and weight of laid eggs were recorded and laying performance, daily egg mass, daily feed intake and feed conversion per kg of eggs and per individual egg calculated. At wks 30, 43, 56 and 69, one egg from each hen (24 from each treatment) was collected to determine eggshell quality, using the EQM system (Technical Services and Supplies, York, England) as described by Krawczyk et al. (2013). At 60 wk of age, one egg from each layer from treatments 6–10 (diets with standard level of Ca) was collected to determine the fatty acid and cholesterol content of the yolk lipids. The measurements were done directly after the eggs were collected.

Cholesterol concentrations were determined in the yolks by gas chromatography (Rong-Zhen et al., 1999). The fatty acid profile of the diets and yolks was determined on a VARIAN 3400 CX gas chromatograph, using helium as a carrier gas, and a 105 m Rtx 2330 column. The injector temperature was 200°C and the detector temperature 240°C. The samples were prepared according to Folch (1957) using methylation with BF3/methanol.

Statistical analysis

The data were subjected to statistical analysis using a completely randomised design, in accordance with the GLM procedure of Statistica 5.0 (StatSoft, Inc., Tulsa, OK, USA). All data were analysed using two-way ANOVA. When significant differences in treatment means were detected by ANOVA (F-test), Duncan's multiple range test was applied to the individual means. Statistical significance was considered to be $P \leq 0.05$.

Results

Production indices

Mean egg production in the experiment throughout the first phase of the laying cycle (26–48 wks of age) was 96.5%; daily mass of eggs, 61.0 g/hen; egg weight, 63.2 g; daily feed intake, 119 g/hen; and feed conversion, 1.95 kg of feed/1 kg of eggs; throughout the second phase (49–70 wks): 90.3%, 58.1 g, 64.5 g, 121 g/hens, and 2.08 kg/kg; and throughout the entire experimental period (25–70 wks): 93.0%, 59.4 g, 63.8 g, 120 g and 2.02 kg/kg, respectively (Tables 2, 3, 4). During the experimental period no dead birds were registered in the treatments. There were no significant differences in the production indices between the hens fed the diet with reduced or standard level of Ca (P>0.05). During the entire experimental period (26–70 wks of age), the addition of the chitosan, probiotic, or herb extracts increased the laying rate (P<0.05), without an effect on egg weight, feed intake and feed conversion ratio.

Table 2. Effects of dietary treatments on performance of hens during first phase of laying cycle, 26–48 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	additives	interaction
1	2	3	4	5	6	7	8	9
Number of eggs produced/100 hens per day	None	96.3	94.6	95.5 a	0.235	0.152	0.001	0.060
	Sodium butyrate	96.0	96.2	96.1 a				
	Probiotic	97.4	95.2	96.3 ab				
	Herb extracts	98.0	97.8	97.9 c				
	Chitosan	96.7	97.9	97.3 bc				
	Mean	96.8	96.4					
Daily mass of eggs (g per hen)	None	61.3	59.9	60.6	0.262	0.778	0.715	0.142
	Sodium butyrate	61.2	61.2	61.2				
	Probiotic	61.0	60.4	60.7				
	Herb extracts	61.0	61.3	61.2				
	Chitosan	60.4	62.6	61.5				
	Mean	61.0	61.1					
Egg weight (g)	None	63.7	63.6	63.5	0.219	0.329	0.445	0.696
	Sodium butyrate	63.8	63.7	63.7				
	Probiotic	62.6	63.4	63.0				
	Herb extracts	62.2	62.7	62.5				
	Chitosan	62.5	63.9	63.2				
	Mean	63.0	63.4					
Daily feed intake (g per hen)	None	119.6	118.8	119.1	0.111	0.328	0.445	0.696
	Sodium butyrate	119.4	119.4	119.4				
	Probiotic	119.7	119.2	119.5				
	Herb extracts	119.2	119.6	119.4				
	Chitosan	118.9	119.6	119.2				
	Mean	119.4	119.3					

Table 2 – contd.

1	2	3	4	5	6	7	8	9
Feed, kg per kg of eggs	None	1.95	1.98	1.97	0.007	0.775	0.692	0.352
	Sodium butyrate	1.95	1.95	1.95				
	Probiotic	1.96	1.98	1.97				
	Herb extracts	1.96	1.95	1.95				
	Chitosan	1.97	1.92	1.94				
	Mean	1.96	1.95					

a, b, c – the values in the rows with different letters differ significantly (P≤0.05).

Table 3. Effects of dietary treatments on performance of hens during second phase of laying cycle, 49–70 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	additives	interaction
Number of eggs produced/100 hens per day	None	85.6	89.4	87.5 a	0.502	0.263	0.038	0.294
	Sodium butyrate	88.8	91.4	90.1 ab				
	Probiotic	91.6	90.9	91.2 b				
	Herb extracts	92.9	91.1	92.0 b				
	Chitosan	89.9	91.3	90.6 b				
	Mean	89.8	90.8					
Daily mass of eggs (g per hen)	None	56.0	57.2	56.6	0.385	0.338	0.329	0.734
	Sodium butyrate	57.8	59.2	58.5				
	Probiotic	58.1	58.2	58.2				
	Herb extracts	59.7	58.7	59.2				
	Chitosan	57.3	59.4	58.3				
	Mean	57.8	58.5					
Egg weight (g)	None	65.4	64.1	64.7	0.259	0.829	0.625	0.623
	Sodium butyrate	65.1	64.9	65.0				
	Probiotic	63.5	64.0	63.7				
	Herb extracts	64.2	64.4	64.3				
	Chitosan	63.7	65.0	64.4				
	Mean	64.4	64.5					
Daily feed intake (g per hen)	None	119.6	119.0	119.3	0.362	0.767	0.088	0.260
	Sodium butyrate	122.6	122.2	122.4				
	Probiotic	121.8	121.5	121.6				
	Herb extracts	122.6	120.3	121.5				
	Chitosan	119.5	121.1	120.8				
	Mean	121.2	121.0					
Feed (kg per kg of eggs)	None	2.14	2.08	2.11	0.012	0.229	0.673	0.916
	Sodium butyrate	2.12	2.06	2.09				
	Probiotic	2.09	2.09	2.09				
	Herb extracts	2.05	2.05	2.05				
	Chitosan	2.09	2.06	2.07				
	Mean	2.10	2.07					

a, b – the values in the rows with different letters differ significantly (P≤0.05).

Table 4. Effects of dietary treatments on performance of hens during entire experimental period, 26–70 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	additives	interaction
Number of eggs produced/100 hens per day	None	90.4	91.9	91.1 a	0.327	0.528	0.001	0.267
	Sodium butyrate	92.1	93.5	92.8 ab				
	Probiotic	94.2	92.8	93.5 b				
	Herb extracts	95.2	94.1	94.7 b				
	Chitosan	92.9	94.2	93.6 b				
	Mean	92.7	93.3					
Daily mass of eggs (g per hen)	None	58.4	58.5	58.4	0.284	0.404	0.435	0.625
	Sodium butyrate	59.4	60.2	59.3				
	Probiotic	59.4	59.2	60.1				
	Herb extracts	60.2	59.9	59.8				
	Chitosan	58.7	60.8	59.7				
	Mean	59.2	59.7					
Egg weight (g)	None	64.5	63.6	64.1	0.222	0.562	0.591	0.594
	Sodium butyrate	64.5	64.4	64.4				
	Probiotic	63.1	63.7	63.4				
	Herb extracts	63.3	63.6	63.4				
	Chitosan	63.1	64.5	63.8				
	Mean	63.7	64.0					
Daily feed intake (g per hen)	None	119.6	118.9	119.3	0.219	0.850	0.080	0.226
	Sodium butyrate	121.1	121.1	121.1				
	Probiotic	120.9	120.4	120.7				
	Herb extracts	121.0	120.0	120.5				
	Chitosan	119.1	121.0	120.1				
	Mean	120.4	120.3					
Feed (kg per kg of eggs)	None	2.05	2.03	2.04	0.008	0.308	0.637	0.913
	Sodium butyrate	2.04	2.01	2.03				
	Probiotic	2.03	2.04	2.04				
	Herb extracts	2.01	2.00	2.00				
	Chitosan	2.03	1.99	2.01				
	Mean	2.01	2.03					

a, b – the values in the rows with different letters differ significantly ($P \leq 0.05$).

Eggshell quality indices

The mean eggshell percentage, averaged across all dietary treatments throughout the experimental period was 11.0% at 30 wk of age; 10.6% at 43 wk of age; 10.9%

at 56 wk of age; and 10.6% at 69 wk of age (Tables 5 to 8). Mean eggshell thickness averaged 384, 374, 389 and 370 μm; eggshell density, 87.7, 85.5, 88.1 and 85.0 mg/cm^2; and eggshell breaking strength, 45.6, 42.7, 42.0 and 36.5 N, respectively, at 30, 43, 56 and 69 wks of age. Egg and eggshell quality parameters at 30 wk of hen age were unaffected by dietary Ca ($P>0.05$), however eggs of older layers (43–69 wks of age) fed the diet with lower Ca level had reduced eggshell percentage, eggshell thickness and eggshell breaking strength ($P<0.05$) (Tables 5 to 8). The studied feed additives had no effect on eggshell quality indices at 30–56 wks of age (Tables 5 to 7), however in older hens, i.e. at 69 wk, diet supplementation with chitosan increased eggshell thickness and breaking strength, while the addition of herb extracts improved the eggshell thickness ($P<0.05$) (Table 8).

Table 5. Effects of dietary treatments on eggshell quality at 30 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	aditives	interaction
Eggshell (%)	None	11.06	10.88	10.97	0.047	0.401	0.570	0.526
	Sodium butyrate	11.16	11.02	11.09				
	Probiotic	11.01	10.91	10.96				
	Herb extracts	11.19	10.98	11.09				
	Chitosan	10.75	11.00	10.88				
	Mean	11.04	10.96					
Eggshell thickness (μm)	None	392	381	386	1.982	0.273	0.108	0.758
	Sodium butyrate	386	386	386				
	Probiotic	373	375	374				
	Herb extracts	394	385	388				
	Chitosan	384	381	382				
	Mean	385	382					
Eggshell density (mg/cm^2)	None	89.7	87.4	88.5	0.526	0.536	0.453	0.302
	Sodium butyrate	89.5	86.1	87.8				
	Probiotic	85.4	87.3	86.3				
	Herb extracts	90.0	88.3	89.1				
	Chitosan	85.9	88.3	87.1				
	Mean	88.1	87.4					
Eggshell breaking strength (N)	None	44.8	43.2	44.1	0.706	0.424	0.557	0.970
	Sodium butyrate	48.3	45.5	46.9				
	Probiotic	45.0	43.8	44.4				
	Herb extracts	47.2	47.0	47.1				
	Chitosan	45.6	45.7	45.7				
	Mean	46.2	45.1					

Table 6. Effects of dietary treatments on eggshell quality at 43 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	additives	interaction
Eggshell (%)	None	10.46	10.70	10.58	0.067	0.030	0.893	0.634
	Sodium butyrate	10.51	10.78	10.65				
	Probiotic	10.54	10.55	10.54				
	Herb extracts	10.37	10.72	10.55				
	Chitosan	10.71	10.72	10.11				
	Mean	10.52 a	10.74 b					
Eggshell thickness (µm)	None	373	369	371	2.760	0.031	0.920	0.128
	Sodium butyrate	373	383	378				
	Probiotic	374	375	374				
	Herb extracts	366	380	373				
	Chitosan	352	390	371				
	Mean	368 a	379 b					
Eggshell density (mg/cm²)	None	84.5	86.6	85.5	0.636	0.127	0.743	0.150
	Sodium butyrate	85.9	87.7	86.9				
	Probiotic	86.4	82.9	84.7				
	Herb extracts	85.3	87.5	86.4				
	Chitosan	81.3	88.2	84.7				
	Mean	84.6	86.6					
Eggshell breaking strength (N)	None	42.0	42.4	42.2	0.735	0.032	0.828	0.322
	Sodium butyrate	41.8	45.9	43.9				
	Probiotic	42.5	41.5	42.0				
	Herb extracts	39.5	46.3	42.9				
	Chitosan	40.0	44.6	42.3				
	Mean	41.2 a	44.1 b					

Table 7. Effects of dietary treatments on eggshell quality at 56 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	additives	interaction
1	2	3	4	5	6	7		
Eggshell (%)	None	10.76	10.86	10.81	0.042	0.019	0.074	0.199
	Sodium butyrate	10.66	11.19	10.93				
	Probiotic	10.79	10.80	10.79				
	Herb extracts	10.99	11.25	11.11				
	Chitosan	10.96	11.00	10.98				
	Mean	10.83 a	11.02 b					
Eggshell thickness (µm)	None	385	390	388	1.820	0.053	0.146	0.095
	Sodium butyrate	378	402	390				
	Probiotic	385	384	385				
	Herb extracts	401	396	398				
	Chitosan	383	393	388				
	Mean	386	393					

Table 7 – contd.

1	2	3	4	5	6	7	8	9
Eggshell density (mg/cm²)	None	89.6	88.7	89.1	0.425	0.318	0.499	0.540
	Sodium butyrate	86.3	90.0	88.2				
	Probiotic	88.8	87.3	87.1				
	Herb extracts	88.6	89.0	88.7				
	Chitosan	87.1	87.7	87.4				
	Mean	87.7	88.5					
Eggshell breaking strength (N)	None	38.6	43.2	40.9	0.541	0.024	0.445	0.631
	Sodium butyrate	42.8	42.3	42.6				
	Probiotic	41.3	43.0	42.1				
	Herb extracts	42.1	45.1	43.6				
	Chitosan	39.2	42.6	40.9				
	Mean	40.8 a	43.2 b					

a, b – the values in the rows with different letters differ significantly (P≤0.05).

Table 8. Effects of dietary treatments on eggshell quality at 69 weeks of age

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		reduced	standard	mean		Ca level	additives	interaction
Eggshell (%)	None	10.26	10.49	10.38	0.041	0.013	0.063	0.817
	Sodium butyrate	10.50	10.61	10.55				
	Probiotic	10.45	10.62	10.53				
	Herb extracts	10.53	10.90	10.71				
	Chitosan	10.62	10.73	10.67				
	Mean	10.47 a	10.70 b					
Eggshell thickness (μm)	None	353	373	363a	1.449	0.001	0.008	0.101
	Sodium butyrate	365	368	366a				
	Probiotic	366	367	367a				
	Herb extracts	369	382	376 b				
	Chitosan	372	377	375 b				
	Mean	365 a	374 b					
Eggshell density (mg/cm²)	None	82.5	85.0	83.8	0.385	0.026	0.337	0.494
	Sodium butyrate	84.8	84.6	84.7				
	Probiotic	83.7	86.1	84.9				
	Herb extracts	84.4	87.8	86.1				
	Chitosan	85.5	85.8	85.7				
	Mean	84.2 a	85.9 b					
Eggshell breaking strength (N)	None	33.4	36.2	34.8 a	0.346	0.028	0.048	0.102
	Sodium butyrate	34.9	36.7	35.8 ab				
	Probiotic	37.4	36.2	36.8 ab				
	Herb extracts	35.8	39.7	37a8 b				
	Chitosan	37.1	37.1	37.1 b				
	Mean	35.7 a	37.2 b					

a, b – the values in the rows with different letters differ significantly (P≤0.05).

Fatty acid and cholesterol content in yolk lipids

Experimental additives included in the diet with standard level of Ca did not significantly affect the fatty acid profile of egg lipids, but diet supplementation with chitosan decreased the content of cholesterol in the yolks (P<0.05) (Tables 9 and 10).

Table 9. Effect of dietary treatment on concentrations of selected fatty acids and cholesterol in egg yolk lipids (%)

Treatment	C16:0	C18:0	C18:1	C18:2 *n-6*	C18:3 *n-3*	C20:4 *n-6*	C20:5 (EPA)	C22:6 (DHA)	Cholesterol (mg/g of yolk)
VI	28.2	7.59	49.0	10.55	0.481	1.64	0.0152	1.08	12.0b
VII	29.5	7.41	47.9	10.20	0.499	1.60	0.0157	1.09	12.2b
VIII	29.6	7.35	48.4	9.71	0.487	1.58	0.0168	1.12	11.9 ab
IX	28.7	7.18	48.5	10.68	0.549	1.59	0.0173	1.07	11.7 ab
X	29.0	7.38	49.4	9.69	0.489	1.56	0.0192	1.05	11.4 a
SEM	0.065	0.465	0.114	0.097	0.094	0.081	0.067	0.523	0.015

a, b – means in columns with different letters differ significantly at P≤0.05.
Treatments: VI–X – diets with standard Ca level; VI – not supplemented, VII – supplemented with sodium butyrate, VIII – supplemented with probiotic bacteria, IX – supplemented with herb extracts mixture, X – supplemented with chitosan.

Table 10. Effect of dietary treatment on concentrations of main groups of fatty acids in egg yolk lipids (%)

Treatment	SFA	UFA	MUFA	PUFA	PUFA *n-6*	PUFA *n-3*	*n-6/n-3*
VI	34.5	63.8	49.9	13.8	12.1	1.57	7.71
VII	37.4	62.6	49.0	13.6	11.9	1.61	7.40
VIII	37.5	62.5	49.4	13.4	11.6	1.63	7.12
IX	36.4	63.6	49.5	14.0	11.9	1.65	7.21
X	37.0	63.0	50.4	13.1	11.5	1.60	7.19
SEM	0.080	0.085	0.065	0.051	0.125	0.227	0.061

a, b, c – means in columns with different letters differ significantly at P≤0.05.
Treatments: VI–X – diets with standard Ca level; VI – not supplemented, VII – supplemented with sodium butyrate, VIII – supplemented with probiotic bacteria, IX – supplemented with herb extracts mixture, X – supplemented with chitosan.

Discussion

Production indices

The obtained performance results are in accordance with our previous study and indicate that 3.20% Ca in the hens' diet is sufficient to maintain good egg production indices (Swiatkiewicz et al., 2015 b). Similar findings were obtained in an earlier experiment by Keshavarz and Nakijima (1993), who found that increasing Ca concentration in the diet (3.50–5.50%) had no effect on the performance of the hens.

Likewise, Pelicia et al. (2009) reported no effect of an increase of Ca dietary level from 3.0 to 4.5% on egg production indices. More recently, An et al. (2016) did not find any influence of dietary Ca level (3.5–4.7% in the diet) on the performance of aged laying hens (70–80 wks of age). Saafa et al. (2008) reported, however, that older layers (58–73 wks of age) require more than 3.5% Ca in the diet for optimal egg production, since an enhancement in Ca dietary concentration to 4.0% significantly increased the laying rate and egg mass, as well as improved the feed conversion ratio.

In this study the supplementation with chitosan, probiotic, or herb extracts positively affected the laying rate during the entire experimental period. Chitosan is soluble in water *N*-deacetylated product of chitin, which is well digested by birds (Swiatkiewicz et al., 2015 c) and its positive influence on the laying rate – as observed in this experiment – may be due to its effect on intestinal morphology and the presence of hypertrophied villi and epithelial cells in birds fed with dietary chitosan (Khambulai et al., 2009). Similarly, in our previous work chitosan increased the laying performance in hens fed a diet containing a high level of DDGS (Swiatkiewicz et al., 2013). The beneficial effect of chitosan on performance parameters was also found in studies with broiler chickens and ducks (Shi et al., 2005; Yuan and Chen, 2012).

The positive effects of probiotic on laying rate observed in this study can be attributed to several mechanisms of probiotic bacteria action in the animal organism, i.e. beneficial alteration in intestinal flora, prevention of proliferation of pathogen bacteria, enhancement of growth-forming lactic acid, improvement of nutrient digestibility, modulation of the immune system, and a decrease of inflammatory reactions (Alloui et al., 2013). The positive influence of the layer diet probiotic in laying hens was also observed by Yörük et al. (2004) who reported that diet supplementation with probiotic increased egg production without an effect on egg quality. Similarly, Loh et al. (2014) found increased egg production in hens fed a diet containing *Lactobacillus plantarum* probiotic bacteria. In the recent experiment by Guo et al. (2017) the positive effect of dietary probiotic (*Bacillus subtilis*) was reflected in improved feed conversion ratio (g of feed/g of produced eggs). In contrast, some other authors did not observe a positive effect of lactic acid-producing bacteria on laying performance (Mahdavi et al., 2005; Mikulski et al., 2012).

The majority of experiments with herb extracts on laying hens were aimed at improving egg quality indices, however Olgun (2016) recently reported the positive effect of herb extracts (a mixture of oils from thyme, black cumin, fennel, anise and rosemary) on egg weight and egg mass, without affecting the laying rate, feed intake and feed conversion ratio. Çabuk et al. (2006) showed that diet supplementation with herb extracts (oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds and citrus peel oils) tends to improve egg production and feed conversion ratio. Akbari et al. (2016) observed an improvement in laying performance, egg mass, and feed conversion ratio in layers under cold stress conditions and fed with an addition of peppermint and thyme essential oils. In contrast, Bozkurt et al. (2012 a) did not find any influence of diet supplementation with essential oil mixture on egg production indices. Ghasemi et al. (2010) observed no differences in egg production and feed conversion ratio between hens fed a diet not supplemented or supplemented with garlic and thyme, however herbs' addition increased egg weight. The inconsistency in the findings on the effects of probiotics and herb extracts on egg production indices was probably due to the differences in the used species and dietary concentrations of these additives.

Eggshell quality indices

In our study the decreased dietary Ca level had negative influence on eggshell quality parameters in older hens. In general, the literature data on the influence of dietary Ca levels on eggshell quality are inconsistent. In their recent study, An et al. (2016) showed that the number of cracked eggs linearly decreased in association with a linear improvement of eggshell breaking strength and thickness when dietary Ca concentration increased from 3.5 to 4.7%. Saafa et al. (2008) found in a study with hens late in the production cycle that eggshell weight, thickness and density was increased when dietary Ca levels were enhanced from 3.5 to 4.0%. The results of the experiment with aged laying hens (58–93 wks of age) showed, similarly to results of our study, that Ca requirements for optimal eggshell quality in hens in the second phase of laying hens are slightly higher than the NRC recommendations (Bar et al., 2002). On the other hand, Cufadar et al. (2011) did not find any effect of Ca level in the diet for moulted layers (3.0–4.2%) on shell breaking strength, relative weight and thickness. Jiang et al. (2013) even reported that laying hens fed diets with high Ca concentrations (4.4%) had decreased shell thickness in comparison with the control group (3.7% Ca). Such inconsistency in the results of studies on the effect of dietary Ca level on eggshell quality may be attributed to different factors that can affect the requirement of layers for Ca, e.g. differences in the strains and age of hens or the content of other nutrients (mainly phosphorus) in the diet.

The results of this experiment showed that such feed additives as chitosan and herb extracts can improve some eggshell quality indices in older hens. The literature data on the effect of chitosan are limited; however, Yoo et al. (2006) reported increased eggshell breaking strength after diet supplementation with this additive. In contrast, in our previous study we did not find any influence of dietary chitosan on the hens' eggshell quality during 26–55 wks of age (Swiatkiewicz et al., 2013). Similarly, no differences in eggshell breaking strength and thickness in layers fed the diet not supplemented or supplemented with chitosan were observed by Meng et al. (2010).

There is some inconsistency in the scientific findings on the efficacy of herb extracts as regards eggshell quality, but in accordance with our results Çabuk et al. (2006) showed that a mixture of essential oils (oregano, laurel leaf, sage leaf, myrtle leaf, fennel seeds, and citrus peel oil) can decrease the number of cracked or broken eggs in older laying hens (54–74 wks of age). Correspondingly, Zhou et al. (2009) reported increased shell breaking strength and tibia bone indices in aged layers fed a diet supplemented with traditional Chinese herbs, indicating that the mechanism of this positive influence of the herbs was possibly associated with minimising structural bone loss and stimulating bone mineral absorption in old osteoporotic hens. Olgun (2016) reported increased eggshell thickness, without differences in eggshell weight and breaking strength, in hens (33 wk of age) fed a diet supplemented with herb extracts. Also, Lokaewmanee et al. (2014) observed that dietary plant extracts (red clover and garlic) increased eggshell breaking strength and indicated that this beneficial influence could probably be attributed to the observed improvements in histological measurements of the small intestine. In contrast, some authors found no positive effect of diet supplementation with herb products in terms of eggshell quality improvement (Bozkurt et al., 2012 b; Swiatkiewicz et al., 2013).

Fatty acids and cholesterol content in yolk lipids

We did not find any effect of used feed additives on fatty acid profile of egg yolk lipids, however diet supplementation with chitosan reduced yolk cholesterol concentration. In accordance with these observations, Nogueira et al. (2003) and Swiatkiewicz et al. (2013) reported that dietary chitosan reduced cholesterol concentration in the yolk lipids. As was shown by Razdan et al. (1997), the hypocholesterolemic effect of dietary chitosan is probably connected with increased binding of bile acids by this additive and, in consequence, with the reduction of the concentration of duodenal bile acids. The positive effect of chitosan on the lipid metabolism was also observed in broiler chickens, and this was connected with a reduction of cholesterol and triglycerides concentration in the blood (Razdan and Petterson, 1994; Razdan et al., 1997) and with a decrease of the abdominal fat pad, without affecting the growth performance parameters (Kobayashi et al., 2002).

Conclusions

Based on the results of this study, it can be concluded that feed additives such as probiotic, herb extracts, or chitosan can beneficially affect laying performance and eggshell quality in aged laying hens, irrespective of Ca dietary level.

References

Abdelqader A., Al-Fataftah A.R., Das G. (2013 a). Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. Anim. Feed Sci. Technol., 179: 103–111.

Abdelqader A., Irshaid R., Al-Fataftah A.R. (2013 b). Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production. Trop. Anim. Health Prod., 44: 1017–1024.

Abe E., Horikawa H., Masumura T., Sugahara M., Kubota M., Suda T. (1982). Disorders of cholecalciferol metabolism in old egg-laying hens. J. Nutr., 112: 436–446.

Akbari M., Torki M., Kaviani K. (2016). Single and combined effects of peppermint and thyme essential oils on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition (6.8±3°C). Internat. J. Biomet., 60: 447–454.

Al-Batshan H.A., Scheideler S.E., Black B.L., Garlich J.D., Anderson K.E. (1994). Duodenal calcium uptake, femur ash and eggshell quality decline with age and increase following molt. Poultry Sci., 73: 1590–1596.

Alloui M.N., Szczurek W., Swiatkiewicz S. (2013). The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. Ann. Anim. Sci., 13: 17–32.

An H.S., Kim D.W., An D.K. (2016). Effects of dietary calcium levels on productive performance, eggshell quality and overall calcium status in aged laying hens. Asian-Australas. J. Anim. Sci., 29: 1477–1482.

AOAC (2000). Official Methods of Analysis, Association of Official Analytical Chemists. 17th Edition. Gaithersburg (USA).
Bar A., Razaphkovsky V., Vax E. (2012). Re-evaluation of calcium and phosphorus requirements in aged laying hens. Brit. Poultry Sci., 43: 261–269.
Bozkurt M., Küçükyilmaz K., Catli A.U., Çınar M., Bintaş E., Cöven F. (2012 a). Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poultry Sci., 91: 1379–1386.
Bozkurt M., Küçükyilmaz K., Pamukçu M., Çabuk M., Alçiçek A., Çatli A.U. (2012 b). Long-term effects of dietary supplementation with an essential oil mixture on the growth and laying performance of two layer strains. Ital. J. Anim. Sci., 11: 23–28.
Çabuk M., Bozkurt M., Alcicek A., Çatlı A.U., Baser K.H. (2006). Effect of a dietary essential oil mixture on performance of laying hens in the summer season. South Afric. J. Anim. Sci., 36: 215–221.
Cufadar Y., Olgun O., Yildiz A.O. (2011). The effect of dietary calcium concentration and particle size on performance, eggshell quality, bone mechanical properties and tibia mineral contents in moulted laying hens. Brit. Poultry Sci., 52: 761–768.
Folch J., Less W., Stanley G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497–509.
Ghasemi R., Zarei M., Torki M. (2010). Adding medicinal herbs including garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) to diet of laying hens and evaluating productive performance and egg quality characteristics. Amer. J. Anim. Vet. Sci., 5: 151–154.
Guo J.R., Dong X.F., Liu S., Tong J.M. (2017). Effects of long-term *Bacillus subtilis* CGMCC 1.921 supplementation on performance, egg quality, and fecal and cecal microbiota of laying hens. Poultry Sci., 96: 1280–1289.
Janssen W.M.M.A. (1989). European Table of Energy Values for Poultry Feedstuffs. 3rd ed. Working Group No. 2 of the European Branch, World's Poultry Science Association, Beekbergen, The Netherlands.
Jiang S., Cui L., Shi C., Ke X., Luo J., Hou J. (2013). Effects of dietary energy and calcium levels on performance, egg shell quality and bone metabolism in hens. Vet. J., 198: 252–258.
Keshavarz K. (2003). A comparison between cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus. Poultry Sci., 82: 1415–1422.
Keshavarz K., Nakajima S. (1993). Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. Poultry Sci., 72: 144–153.
Khambulai O., Yamauchi K., Tangtaweewipat T., Cheva-Isarakul B. (2009). Growth performance and intestinal histology in broiler chickens fed with dietary chitosan. Brit. Poultry Sci., 50: 592–597.
Kobayashi S., Terashima Y., Itoh H. (2002). Effects of dietary chitosan on fat deposition and lipase activity in digesta in broiler chickens. Brit. Poultry Sci., 43: 270–273.
Koreleski J., Swiatkiewicz S. (2004). Calcium from limestone meal and grit in laying hen diets – effect on performance, eggshell and bone quality. J. Anim. Feed Sci., 13: 635–645.
Krawczyk J., Sokolowicz Z., Swiatkiewicz S., Sosin-Bzducha E. (2013). Effect of outdoor access and increased amounts of local feed materials in the diets of hens covered by the gene-pool protection programme for farm animals in Poland on quality of eggs during peak egg production. Ann. Anim. Sci., 13: 327–339.
Lichovnikova M. (2007). The effect of dietary calcium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens. Brit. Poultry Sci., 48: 71–75.
Loh T.C., Choe D.W., Foo H.L., Sazili A.Q., Bejo M.H. (2014). Effects of feeding different postbiotic metabolite combinations produced by *Lactobacillus plantarum* strains on egg quality and production performance, faecal parameters and plasma cholesterol in laying hens. BMC Vet. Res., 149: 1–9.
Lokaewmanee K., Yamauchi K.E., Komori T., Saito K. (2014). Eggshell quality, eggshell structure and small intestinal histology in laying hens fed dietary Pantoea-6® and plant extracts. Ital. J. Anim. Sci., 13: 332–339.

Mabe I., Rapp C., Bain M.M., Nys Y. (2003). Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poultry Sci., 82: 1903–1913.

Mahdavi A.H., Rahman A.R., Pourreza J. (2005). Effect of probiotic supplements on egg quality and laying hen's performance. Internat. J. Poultry Sci., 4: 488–492.

Meng Q.W., Yan L., Ao X., Jang H.D., Cho J.H., Kim I.H. (2010). Effects of chito-oligosaccharide supplementation on egg production, nutrient digestibility, egg quality and blood profiles in laying hens. Asian-Australas. J. Anim. Sci., 23: 1476–1481.

Mikulski D., Jankowski J., Naczmanski J., Mikulska M., Demey V. (2012). Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poultry Sci., 91: 2691–2700.

Nogueira C.M., Zapata J.F.F., Fuentes M.F.F., Freitas E.R., Craveiro A.A., Aguiar C.M. (2003). The effect of supplementing layer diets with shark cartilage or chitosan on egg components and yolk lipids. Brit. Poultry Sci., 44: 218–223.

Nys V. (1999). Nutritional factors affecting eggshell quality. Czech J. Anim., 44: 135–143.

Nys Y. (2001). Recent developments in layer nutrition for optimising shell quality. Proc. 13th Europ. Symp. Poultry Nutr., Blankenberge, Belgium, pp. 45–52.

Olgun O. (2016). The effect of dietary essential oil mixture supplementation on performance, egg quality and bone characteristics in laying hens. Ann. Anim. Sci., 16: 1115–1125.

Pastore S.M., Gomes P.C., Rostagno H.S., Albino L.F.T., Calderano A.A., Vellasco C.R., Da Silva V.G, Almeida R.L.D. (2012). Calcium levels and calcium: available phosphorus ratios in diets for white egg layers from 42 to 58 weeks of age. Braz. J. Anim. Sci., 41: 2424–2432.

Pelicia K., Garcia E., Mori C., Faitarone A.B.G., Silva A.P., Molino A.B., Vercese F., Berto D.A. (2009). Calcium levels and limestone particle size in the diet of commercial layers at the end of the first production cycle. Braz. J. Poultry Sci., 11: 87–94.

Razdan A., Pettersson D. (1994). Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentration in broiler chickens. Brit. J. Nutr., 72: 277–288.

Razdan A., Pettersson D., Pettersson J. (1997). Broiler chicken body weights, feed intakes, plasma lipid and small-intestinal bile acid concentration in response to feeding chitosan and pectin. Brit. J. Nutr., 74: 283–291.

Roland D.A. (1988). Research note: egg shell problems: estimates of incidence and economic impact. Poultry Sci., 67: 1801–1803.

Rong-Zhen Z., Long L., Shu-Tao L., Ru-Ming C., Ping-Fan R. (1999). An improved method of cholesterol determination in egg yolk by HPLC. J. Food Bioch., 23: 351–361.

Saafa H., Serrano M.P., Valencia D.G., Frikha M., Jiménez-Moreno E., Mateos G.G. (2008). Productive performance and egg quality of brown egg-laying hens in the late phase of production as influenced by level and source of calcium in the diet. Poultry Sci., 87: 2043–2051.

Sengor E., Yardimci M., Cetingul S., Bayram I., Sahin H., Dogan I. (2007). Effects of short chain fatty acid (SCFA) supplementation on performance and egg characteristics of old breeder hens. South Afric. J. Anim. Sci., 37: 158–163.

Shi B.L., Li D.F., Piao X.S., Yan S.M. (2005). Effects of chitosan on growth performance and energy and protein utilisation in broiler chickens. Brit. Poultry Sci., 46: 516–519.

Sobczak A., Kozłowski K. (2015). The effect of a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 on egg production and physiological parameters of laying hens. Ann. Anim. Sci., 15: 711–723.

Swiatkiewicz S., Arczewska-Wlosek A. (2012). Prebiotic fructans and organic acids as feed additives improving mineral availability. World's Poultry Sci. J., 68, 269–279.

Swiatkiewicz S., Koreleski J., Arczewska A. (2010). Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. Czech J. Anim. Sci., 55: 294–306.

Swiatkiewicz S., Arczewska-Wlosek A., Krawczyk J., Puchala M., Jozefiak D. (2013). Effects of selected feed additives on the performance of laying hens given a diet rich in maize dried distiller's grains with solubles (DDGS). Brit. Poultry Sci., 54: 478–485.

Swiatkiewicz S., Arczewska-Wlosek A., Krawczyk J., Puchala M., Jozefiak D.

(2015 a). Dietary factors improving eggshell quality: an updated review with special emphasis on microelements and feed additives. Worlds' Poultry Sci. J., 71: 83–94.
Swiatkiewicz S., Arczewska-Wlosek A., Krawczyk J., Puchala M., Jozefiak D. (2015 b). Effects on performance and eggshell quality of particle size of calcium sources in laying hens' diets with different Ca concentrations. Arch. Anim. Breed., 58: 301–307.
Swiatkiewicz S., Swiatkiewicz M., Arczewska-Wlosek A., Jozefiak D. (2015 c). Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. J. Anim. Physiol. Anim. Nutr., 99: 1–12.
Yoo J.S., Kim J.D., Cho J.H., Chen Y.J., Kim H.J., Min B.J., Kang D.K., Kim I.H. (2006). The effect of natural mineral complex and chitosan supplementation on egg production and characteristics in laying hens. Korean J. Poultry Sci., 33: 309–316.
Yörük M.A., Gül M., Hayirli A., Macit M. (2004). The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Sci., 83: 84–88.
Yuan S., Chen H. (2012). Effects of dietary supplementation of chitosan on growth performance and immune index in ducks. Afric. J. Biotechnol., 11: 3490–3495.
Zhou Z.L., Deng Y.F., Tao Q.S., Hu Y.F., Hou J.F. (2009). Effects of Gushukang, a Chinese herbal medicine, on bone characteristics and osteoporosis in laying hens. Poultry Sci., 88: 2342–2345.

Received: 30 X 2017
Accepted: 20 XI 2017