Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids

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ABSTRACT: The aim of the study was to evaluate the effect of organic acids and the prebiotic fructans on egg production and eggshell quality when added to the layer diet with different levels of calcium and phosphorus. The experiment was carried out on 168 Bovans Brown hens, allocated to 14 groups of 12 replications. Each hen (replication) was kept in an individual cage 40 cm × 40 cm in size. A 2 × 7 factorial arrangement, with two dietary levels of calcium and phosphorus (normal – 3.70% Ca, 0.65% P, and reduced – 3.25% Ca, 0.60% P) and with diets supplemented by selected additives (none, 0.75% inulin, 0.75% oligofructose, 0.50% volatile fatty acids (VFA), 0.25% medium chain fatty acid (MCFA), 0.30% VFA + 0.20% MCFA, 0.75% inulin + 0.50% VFA) was used. The experiment was carried out over 34 weeks, from the age of 26 to 70 weeks. There were no statistically confirmed effects of the factors studied in this experiment on egg performance, i.e. laying rate, egg mass, feed intake and feed conversion. Reducing the dietary levels of Ca and P significantly decreased eggshell percent, thickness, density and breaking strength. The additives used had a considerable effect on eggshell quality at 46, 58 and 70 weeks of age, and these positive effects were most pronounced in the case of inulin and MCFA. There was no significant interaction between Ca and P dietary levels and the additives used. It was thus concluded that selected feed additives which lower the pH of the diet and intestinal content can beneficially influence eggshell quality in older high-producing laying hens.

Keywords: laying hens; egg production; eggshell quality; organic acids; prebiotic fructans

Eggshell quality is one of the most important issues in the poultry industry, influencing the economic profitability of egg production and hatchability. High breaking strength of eggshell and absence of shell defects are essential for protection against the penetration of pathogenic bacteria such as *Salmonella* sp. into eggs. It has been estimated that eggs with damaged shells account for 6–10% of all eggs produced, which leads to great economic losses (Washburn, 1982; Roland, 1988). One of the main concerns is a decrease in eggshell quality as the hen ages, due to an increase in egg weight without an increase in the amount of calcium carbonate deposited in the shells. For this reason, the incidence of cracked eggs could even exceed 20% at the end of the laying period (Nys, 2001).

Most of the studies on the effects of nutrition on eggshell quality in laying hens have been focused on macrominerals (Ca, P) and vitamin D_3 (Nys, 1999). Supplying the hen with an optimal Ca intake is the most crucial 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

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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 (Koreleski and Swiatkiewicz, 2004).

There is also some evidence that feed additives increasing the availability of Ca and other minerals may improve hen eggshell quality. The results of some studies carried out on rats, broiler chickens and pigs have indicated that organic acids may improve the utilization of minerals in monogastric animals (Lutz and Scharrer, 1991; Radclife et al., 1998; Boling et al., 2000; Mroz et al., 2000; Omogbenigun et al., 2003; Liem et al., 2008). One of the factors the mechanism of this effect is connected with is the reduction of intestinal pH, which leads to an increase in the activity of digestive enzymes (accelerated conversion of pepsinogen to pepsin) and in the solubility of minerals. Some experiments with layers and old broiler breeder hens have demonstrated that organic acids can have a positive effect on laying performance and eggshell quality (Park et al., 2002; Yesilbag and Colpan, 2006; Sengor et al., 2007; Soltan, 2008). In the recent study with older laying hens (from 75 to 80 weeks of age) organic acids beneficially affected soft-shell + broken egg production, feed conversion ratio and IgY concentration in yolks, but had no influence on laying rate and eggshell strength and thickness (Park et al., 2009). The addition of acidifiers (phosphoric acid and citric acid) to the diet for broilers lowered the pH of the crop and gizzard content, however, there were no statistically significant effects of acids on performance parameters (Andrys et al., 2003).

Prebiotics are defined as non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one, or a limited number, of bacteria in the colon (Gibson and Roberfroid, 1995). Inulin and oligofructose, i.e. the fructans that naturally occur in many plants, are considered to have strong prebiotic properties. They are not digested by the enzymes of monogastric animals, so they are fully available to fermentation by intestinal microflora and may selectively stimulate the growth of lactic acid bacteria, mainly *Bifidobacterium*. Inulin is a long-chain fructan containing up to 60 fructose molecules, whereas oligofructose is a partial enzymatic hydrolysate of inulin, with a chain length between 3 and 8 (Chen et al., 2005b). The positive effect of inulin or fructooligosaccharides on the utilization of Ca and other minerals was observed mainly in model experiments with rats (Delzenne et al. 1995; Ohta et al., 1995; Morohashi et al., 1998; Kruger et al., 2003; Zafar et al., 2004; Coudray et al., 2005; Coudray et al., 2006; Demigne et al., 2008). Added to the diet for broilers, inulin increased the length of intestinal villus (Rehman et al., 2007), which might stimulate the absorption of minerals. In laying hens, oligofructose or inulin improved egg performance, mineral utilization, eggshell percent and eggshell breaking strength, they increased the serum pancreatic amylase activity and decreased the concentration of cholesterol in yolk, without affecting the interior egg quality (Chen and Chen, 2004; Chen et al., 2005a,b,c).

The objective of the present study was to evaluate the effect of addition of inulin, oligofructose, volatile fatty acids or medium-chain fatty acids to the diet with two levels of calcium and phosphorus on egg performance and eggshell quality in high-producing layer hens.

MATERIAL AND METHODS

Birds, diets and management

The Krakow Local Ethic Committee for Experiments with Animals approved all the experimental procedures relating to the use of live animals. A total of 168 Bovans Brown hens at 18 weeks of age, obtained from a commercial source, were placed in individual cages on the wire-mesh floor under controlled climate conditions in a poultry house of the Experimental Station of the National Research Institute of Animal Production. The cage dimensions were 40 × 40 cm, equalling 1 600 cm^2 of the floor space in total. During the pre-experimental period, a commercial laying hen diet was offered for *ad libitum* consumption (17% crude protein, 2 770 kcal/kg AME_N, 3.70% Ca and 0.38% available P).

At 26 weeks of age, the hens were randomly assigned to one of the 14 dietary treatments, with 12 individually caged layers for each treatment. During the experiment, the hens were provided with water and feed *ad libitum*, and they were exposed to a 14 L:10 D lighting schedule with a light intensity of 10 lux.

A 2 × 7 factorial arrangement, with two dietary levels of Ca and P and with supplementation of experimental additives to diets, was used. The basal

Table 1. Composition of experimental diets (%)

Ingredients	Control	Reduced level of Ca and P
Maize	51.42	53.92
Wheat	12.00	12.00
Soybean oil meal	23.60	23.00
Rapeseed oil	1.40	0.70
Limestone	9.40	8.40
Monocalcium phosphate	1.25	1.05
NaCl	0.30	0.30
DL-methionine	0.13	0.13
Vitamin-mineral premix[1]	0.50	0.50
Calculated nutrient content[2]		
Crude protein (%)	17.00	17.00
Metabolizable energy[3] (MJ/kg)	11.60	11.60
Lys (%)	0.82	0.82
Met (%)	0.39	0.39
Ca (%)	3.70	3.25
P (%)	0.65	0.60
P available (%)	0.40	0.34
Analyzed		
Ca (%)	3.90	3.25
P (%)	0.66	0.60

[1]the premix provided per 1 kg of diet: vitamin A 10 000 IU; vitamin D_3 3 000 IU; vitamin E 50 IU; vitamin K_3 2 mg; vitamin B_1 1 mg; vitamin B_2 4 mg; vitamin B_6 1.5 mg; vitamin B_{12} 0.01 mg; Ca-pantothenate 8 mg; niacin 25 mg; biotin 0.1 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 from tables of feed composition on the basis of component nutrient content

[3]according to the European Table of Energy Values for Poultry Feedstuffs (Janssen, 1989) as a sum of ME content of feed components calculated on the basis of nutrient content

experimental diets (Table 1) contained normal (3.70 and 0.65%) or lowered (3.25 and 0.60%) levels of Ca and P. These diets were either unsupplemented or supplemented with additives as follows: 0.75% inulin (BENEO™ IPS, Orafti, Belgium), 0.75% oligofructose (Beneo™ OPS, Orafti, Belgium), 0.50% VFA (0.20% formic, 0.15% propionic and 0.15% acetic acid), 0.25% MCFA (0.125% caproic and 0.125% capric acid), 0.30% VFA + 0.20% MCFA or 0.75% inulin + 0.50% VFA.

The experimental diets were fed to the hens from the 25th to the 70th week. The nutrient content of the diets was calculated in accordance with the chemical composition of raw feedstuffs, and the metabolizable energy value on the basis of equations from European Tables (Janssen, 1989). Samples of feed components were analyzed, using standard methods (AOAC, 1990), for moisture (method 930.15), crude protein (984.13), crude fat (920.39) and ash (942.05). Amino acids were analyzed in acid hydrolysates, after initial performic acid oxidation of sulphur amino acids and after alkaline hydrolysis of tryptophan (AOAC, 1990; method 982.30). The Ca content was analyzed by flame atomic absorption spectrophotometry (AOAC, 1990; method 968.08) and total P content by colorimetry, using the molybdo-vanadate method (AOAC, 1990; method 965.17).

Measurements

During the experiment, the number and weight of eggs were registered daily, feed consumption was recorded monthly, and egg production, egg mass, daily feed intake and feed conversion (feed consumed per 1 g of produced egg mass) were calculated.

At 34, 46, 58 and 70 weeks, one egg from each hen (i.e. 12 eggs from each treatment) was collected to determine eggshell quality indices, i.e. eggshell percent, eggshell thickness and eggshell density. The eggs were analyzed using semi-automated egg quality equipment (QCM+, Technical Services and Supplies (TSS), York, UK). Shell thickness was measured near the equator of the egg, using an electronic micrometer (QCT device, TSS, York, UK). Eggshell density (dried shell weight per unit of shell area, mg/cm^2) was calculated by Eggware software (TSS, York, UK). Further 12 eggs from each treatment were collected for the measurement of eggshell breaking strength, using an Instron Testing Machine, Model 5542 (Instron Ltd., High Wycombe, England), equipped with a 500 Newton load cell. The eggs were compressed at a constant crosshead speed of 10 mm/min, and breaking strength was determined at the moment of the eggshell fracture.

Statistical analysis

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

RESULTS

Laying performance

The mean egg production, averaged across all the dietary treatments throughout the experimental period (hen age 26–70 weeks) was 93.4%; egg weight 63.5 g; daily egg mass 59.4 g/hen; daily feed consumption 121 g/hen; and feed conversion 2.02 kg of feed/1 kg of eggs (Table 2). The mean mortality during the experiment was low and amounted to 0.6% (one dead layer in the group with reduced level of dietary Ca and P and without additives was noted). The experimental factors (dietary level of Ca and P and dietary supplementation of selected additives) had no significant effect on laying performance parameters, as compared with the control group ($P > 0.05$).

Eggshell quality

The mean eggshell percent, averaged across all the dietary treatments throughout the experimental period, was 10.8% at 34 weeks of age; 11.0% at 46 weeks of age; 10.9% at 58 weeks of age; and 10.6% at 70 weeks of age (Table 3). The mean eggshell thickness was 382, 392, 391 and 380 μm (Table 4); eggshell density 87.4, 89.3, 88.3 and 85.5 mg/cm^2 (Table 5); and eggshell breaking strength 33.5, 34.6, 33.2 and 32.3 N (Table 6), respectively at 34, 46, 58 and 70 weeks of age. Reducing the dietary levels of Ca and P significantly decreased the eggshell percent in hens at 34, 58 and 70 weeks of age, eggshell thickness at 34, 46, 58 and 70 weeks of age, eggshell density at 34, 58 and 70 weeks of age and eggshell breaking strength at 34, 46, 58 and 70 weeks of age.

At 36 weeks of age, there were no statistically confirmed effects of the additives used in this experiment on eggshell percent, thickness, density and breaking strength, but in older hens some additives positively affected the eggshell quality. In 46-weeks-old hens, eggshell breaking strength was improved after the inclusion of inulin and MCFA in the diet (Table 6). At 58 weeks of age, eggshell percent and density were significantly increased by inulin, VFA, MCFA, VFA + MCFA and inulin + VFA addition (Tables 3 and 5). At 70 weeks of age, eggshell percent was significantly improved by MCFA (Table 3), eggshell density by VFA and MCFA (Table 5) and eggshell breaking strength by inulin, oligofructose, VFA, MCFA, VFA + MCFA, and inulin + VFA (Table 6).

DISCUSSION

Laying performance

In our study, there were no statistically confirmed effects of the experimental factors (dietary level of

Table 2. Effects of experimental factors on egg production from 25 to 70 weeks of age

Additives used	Laying rate (%)			Daily egg mass (g/hen/day)			Egg weight (g)			Feed consumption (g/hen/day)			Feed conversion (kg/1 kg of eggs)		
	dietary levels of Ca and P														
	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean
None	94.3	90.5	92.4	60.2	57.3	58.7	63.8	63.2	63.5	121	121	121	2.02	2.11	2.06
Inulin	93.8	93.4	93.6	60.7	59.9	60.3	64.6	64.2	64.4	121	121	121	2.00	2.01	2.01
Oligofructose	93.7	92.6	93.1	60.3	59.3	59.8	64.4	64.0	64.2	122	121	121	2.02	2.04	2.03
VFA	94.3	91.1	93.2	60.1	58.2	59.2	63.7	63.2	63.5	120	121	121	2.01	2.07	2.04
MCFA	95.3	94.2	94.7	60.5	60.3	60.4	63.6	64.0	63.8	121	121	121	2.00	2.00	2.00
VFA + MCFA	92.8	94.3	93.6	60.2	60.6	60.4	64.9	64.2	64.5	120	120	120	2.00	1.99	1.99
Inulin + VFA	91.7	93.5	92.6	59.2	60.3	59.7	64.6	64.4	64.5	120	121	121	2.04	2.01	2.02
Mean	93.7	92.9		60.2	59.4		64.2	63.9		121	121		2.01	2.03	
SEM		0.296			0.303			0.231			0.211			0.011	
Effect of															
Level of Ca and P		NS			NS			NS			NS			NS	
Additives		NS			NS			NS			NS			NS	
Interaction		NS			NS			NS			NS			NS	

NS – $P > 0.05$

Table 3. Effects of experimental factors on eggshell percent (%)

Additives used	Hen's age (in weeks)											
	34			46			58			70		
	dietary levels of Ca and P											
	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean
None	10.9	10.2	10.6	11.0	10.4	10.7	10.7	10.5	10.6^{a}	10.4	10.3	10.4^{a}
Inulin	11.0	10.5	10.8	11.3	11.0	11.1	11.2	11.0	11.1^{c}	10.8	10.6	10.7^{ab}
Oligofructose	10.9	10.8	10.8	11.1	11.0	11.0	10.8	10.5	10.7^{ab}	10.6	10.2	10.4^{a}
VFA	11.0	10.5	10.7	11.2	10.9	11.0	11.2	10.8	11.0^{bc}	11.0	10.5	10.7^{ab}
MCFA	11.3	10.9	11.1	11.1	11.1	11.1	11.2	10.9	11.1^{c}	11.0	10.8	10.9^{b}
VFA + MCFA	11.2	10.5	10.8	11.1	11.0	11.0	11.0	10.8	10.9^{bc}	10.6	10.4	10.5^{ab}
Inulin + VFA	11.1	10.9	11.0	10.6	11.3	10.9	10.8	11.1	11.0^{bc}	10.5	10.6	10.5^{ab}
Mean	11.0	10.6		11.1	10.9		11.0	10.8		10.7	10.5	
SEM		0.056			0.069			0.048			0.045	
Effect of												
Level of Ca and P		**			NS			*			*	
Additives		NS			NS			*			*	
Interaction		NS			NS			NS			NS	

^{a,b,c}values in the columns with different letters differ significantly ($P \le 0.05$); NS – $P > 0.05$; $^{*}P \le 0.05$; $P \le 0.01$

Ca and P and dietary supplementation of selected additives) on laying performance. Similarly to our findings, Yildiz et al. (2006) reported no effect of inulin added to the diet in the form of dried Jerusalem artichoke (*Helianthus tuberosus* L.) on laying rate, feed consumption and feed conversion. In contrast, Chen et al. (2005b) found that the inclusion of 1% inulin or oligofructose in the layer diet improved egg production and feed conversion.

The effect of organic acids (a mixture of formic and propionic acids) on laying performance was studied in an experiment with Lohman layers conducted by Yesilbag and Colpan (2006). Similarly to our results, they found no effect of organic acids on feed consumption and feed conversion; however they observed an improved laying rate at 24–28 and 36–38 weeks of age as a result of the inclusion of 0.5–1.5% organic acids into the diet. Also Gama et al. (2000) showed that the addition of organic acids into layer diet for 4 or 8 weeks improved egg production. Soltan (2008) reported the beneficial influence of organic acids (formic acid and salts of butyric, propionic and lactic acids) on average egg production (from 54 to 70 weeks of age) at the highest inclusion level (780 ppm), but not when the lower inclusion levels (260 or 520 ppm) were used. The positive effect of acetic acid on laying performance was also observed in hens reared under conditions of heat stress (Kadim et al., 2008).

Eggshell quality

In our experiment, the tendency toward a decline in eggshell quality as the hen ages was observed (the mean eggshell breaking strength from 33.5 N at 34 weeks of age and to 32.3 N at 70 weeks of age). A similar tendency toward the negative effect of the hen's age on eggshell quality was reported by Al-Batshan et al. (1994), De Ketelaere et al. (2002) and Swiatkiewicz and Koreleski (2008), among others. In White Leghorn hens in the late laying phase (55 to 70 weeks of age), eggshell quality, measured as shell specific gravity, was significantly lower than in younger birds (Castillo et al., 2004).

Table 4. Effects of experimental factors on eggshell thickness (μm)

Additives used	Hen's age (in weeks)											
	34			46			58			70		
	dietary levels of Ca and P											
	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean
None	387	360	373	386	372	378	386	379	382	378	363	371
Inulin	397	364	381	407	390	398	407	391	399	390	376	382
Oligofructose	391	376	383	397	394	396	392	377	384	383	378	381
VFA	383	377	380	400	381	390	398	391	395	383	382	382
MCFA	386	374	380	394	394	394	399	392	395	388	386	387
VFA + MCFA	395	362	378	402	387	395	398	378	388	381	376	379
Inulin + VFA	397	383	390	387	395	391	387	398	393	381	381	381
Mean	391	373		396	388		395	387		384	377	
SEM	2.73				1.98			1.80			1.53	
Effect of												
Level of Ca and P		**			*			*			*	
Additives		NS			NS			NS			NS	
Interaction		NS			NS			NS			NS	

NS – $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$

In this study, reducing the dietary levels of Ca and P negatively affected eggshell quality parameters throughout the whole experimental period (at 34, 46, 58 and 70 weeks of age). Similar results were obtained in a study conducted on Hy-Line layers, where increasing the dietary Ca level from 3.1 to 3.7% resulted in a significant linear improvement of eggshell quality (Sohail and Roland, 2000). The significant effect of the dietary Ca level on the eggshell was also observed by Castillo et al. (2004), who noticed that shell specific gravity was greater in hens fed a diet with 3.83% Ca than in those receiving 2.96% Ca; however there were no significant differences between the Ca levels of 3.22 and 3.83%. Safaa et al. (2008) reported that eggshell percent, thickness and density significantly increased when the dietary level of Ca was raised from 3.5 to 4.0%. In a study carried out on white Lohman hens, increasing the Ca level from 3.0 to 3.8 had a positive effect on eggshell weight (Narvaez-Solarte et al., 2006). In contrast, Rama Rao et al. (2003) found no beneficial effect on eggshell weight and thickness when the dietary Ca content was increased from 3.25 to 4.5%. In our experiment, we found a positive effect of the prebiotic fructans on some eggshell quality parameters (eggshell percent, density and breaking strength) in older hens (at 46, 58 and 70 weeks of age). Experimental data on the effect of the prebiotic fructans on eggshell quality are scarce. The results obtained by Chen and Chen (2004) were similar to our findings; they reported that supplementation of 1% oligofructose or inulin to the diet significantly increased eggshell percent and eggshell breaking strength, Ca concentration in serum, and crude ash, Ca and P levels in bones of the tibia. In moulted hens, supplementation of fructooligosaccharides (0.75%) to a lucerne diet prevented a decrease in the tibia and femur breaking strength and in mineral content of the tibia during moulting (Kim et al., 2006). The authors of this study have concluded that, probably due to their beneficial effect on Ca absorption, fructooligosaccharides have the potential to maintain the bone strength, which is often reduced by the structural bone loss during moulting. In contrast to the results of our experiment, Yildiz et al. (2006) found no

Table 5. Effects of experimental factors on eggshell density (mg/cm^2)

Additives used	Hen's age (in weeks)											
	34			46			58			70		
	dietary levels of Ca and P											
	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean
None	87.0	83.0	85.0	88.8	81.7	85.2	85.9	84.6	85.2^{a}	84.3	82.3	83.3^{a}
Inulin	87.2	84.3	85.8	92.9	87.0	90.0	92.9	89.3	90.7^{c}	86.0	85.9	86.0^{ab}
Oligofructose	87.0	86.7	86.9	88.4	90.2	89.3	87.2	86.4	86.8^{ab}	85.9	83.8	84.8^{ab}
VFA	86.7	84.2	85.4	90.0	89.4	89.7	91.3	87.3	89.3^{bc}	88.5	84.8	86.6^{b}
MCFA	89.5	85.4	87.5	91.9	91.5	91.7	90.2	87.7	88.9^{bc}	88.4	87.4	87.9^{b}
VFA + MCFA	88.6	84.0	86.3	90.7	87.2	89.0	90.4	86.7	88.6^{bc}	86.0	84.4	85.2^{ab}
Inulin + VFA	88.4	87.3	87.9	88.7	92.3	90.5	88.7	88.8	88.8^{bc}	86.4	83.2	84.8^{ab}
Mean	87.8	85.0		90.2	88.5		89.4	87.2		86.5	84.5	
SEM		0.616			0.595			0.449			0.387	
Effect of												
Level of Ca and P		*			NS			*			*	
Additives		NS			NS			*			*	
Interaction		NS			NS			NS			NS	

[a,b,c]values in the columns with different letters differ significantly ($P \leq 0.05$); NS – $P > 0.05$, *$P \leq 0.05$

Table 6. Effects of experimental factors on eggshell breaking strength (N)

Additives used	Hen's age (in weeks)											
	34			46			58			70		
	dietary levels of Ca and P											
	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean	normal	reduced	mean
None	33.5	31.4	32.4	33.5	30.9	32.2[a]	31.7	30.4	31.0	30.5	29.7	30.1[a]
Inulin	35.2	32.0	33.6	37.9	34.4	36.2[b]	36.6	32.0	34.3	34.0	31.1	32.8[b]
Oligofructose	33.8	32.9	33.3	35.4	34.4	34.9[ab]	34.3	31.7	33.0	33.7	31.5	32.6[b]
VFA	34.5	31.9	33.2	35.1	33.2	34.1[ab]	36.3	31.9	34.1	34.2	31.9	33.1[b]
MCFA	35.8	33.1	34.5	37.8	34.9	36.4[b]	36.1	32.4	34.3	34.3	32.9	33.6[b]
VFA + MCFA	36.7	31.4	34.1	35.8	32.7	34.3[ab]	35.0	31.2	33.0	33.2	31.9	32.5[b]
Inulin + VFA	34.8	32.8	33.8	33.7	34.5	34.1[ab]	33.2	33.3	33.3	32.2	31.8	32.0[ab]
Mean	34.9	32.2		35.6	33.6		34.7	31.8		33.1	31.6	
SEM		0.465			0.352			0.363			0.276	
Effect of												
Level of Ca and P		**			**			**			**	
Additives		NS			*			NS			*	
Interaction		NS			NS			NS			NS	

[a,b]values in the columns with different letters differ significantly ($P \leq 0.05$); NS – $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$

statistically confirmed effect of inulin from dried Jerusalem artichoke on the weight, thickness and breaking strength of eggshells during a 16-week trial with layers.

The positive effect of the prebiotic fructans, especially inulin, on eggshell quality, observed in this study, is probably connected with the stimulation of mineral availability. According to Scholz-Ahrens et al. (2007), the mechanism of the positive effect of fructans on mineral utilization is complex and can be attributed to such factors as the high solubility of minerals because of the increased production of short-chain fatty acids by probiotic bacteria through an increased supply with substrate (fructans), alteration of the intestinal mucosa and increase of the absorption surface by means of the beneficial effect of bacterial fermentation products on the proliferation of enterocytes, increased expression of Ca-binding proteins, release of bone modulating factors, degradation of phytates by probiotic bacteria enzymes and overall improvement of the gut health.

In this study, the addition of MCFA and, to a lesser extent, VFA, had a positive influence on eggshell characteristics, i.e. eggshell percent, density and breaking strength, at 46, 58 and 70 weeks of age. This influence can probably be attributed to an increased availability of Ca and P, brought about by a decrease in pH in the upper part of the intestinal tract and the stimulating effect of organic acids on the villus height, which was observed in broilers by Garcia et al. (2007) and Senkoylu et al. (2007). It has also been proposed that organic acids (citric acid) improved Ca availability by chelating Ca and reducing the formation of insoluble Ca-phytate-complexes (Boling et al., 2000). Abdel-Fattah et al. (2008) reported that chicks fed a diet supplemented with organic acids had significantly higher Ca and P blood concentrations, which the authors attributed to the lowering of intestinal pH and the increase in the absorption of these macroelements by the utilization of these acids.

The results of the present study are similar to those reported by Sengor et al. (2007), who in an experiment with old breeder White Bovans hens found that eggshell breaking strength was increased after the inclusion of short-chain fatty acids (VFA) in the diet. The authors also reported that VFA decreased the number of dirty, cracked and misshapen eggs. Soltan (2008) stated that organic acids (a mixture of formic acid and salts of butyric, propionic and lactic acids) added to the diet in the amount of 780 ppm improved eggshell thickness at 70 weeks of age and reduced the number of broken eggs, but had no effect on eggshell weight. The author indicated that the observed improvement in eggshell quality was connected with an increase in Ca concentration in serum, which can be attributed to the beneficial effect of organic acids on Ca absorption (Soltan, 2008). Orban et al. (1993) reported that high doses of ascorbic acid (2 000 or 3 000 ppm) improved the specific gravity of eggs, which was connected with the increased concentration of Ca in the blood. In contrast, in their experiment with Lohman layers, Yesilbag and Colpan (2008) found no effect of formic and propionic acids on eggshell thickness and eggshell breaking strength.

In conclusion, the results of this study indicate that selected feed additives which lower the pH of the diet and intestinal content could have a positive effect on the eggshell quality in older (from 46 to 70 weeks of age), high-producing laying hens fed a diet with a normal or reduced level of Ca and P. A significant improvement in shell quality was obtained by the use of inulin, oligofructose, volatile fatty acids and medium-chain fatty acids.

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