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THE EFFECT OF A PROBIOTIC PREPARATION CONTAINING *BACILLUS SUBTILIS* ATCC PTA-6737 ON EGG PRODUCTION AND PHYSIOLOGICAL PARAMETERS OF LAYING HENS

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Abstract

A total of 288 Lohmann Brown laying hens were randomly divided into two groups (9 replicates of 16 birds each). The hens were housed in three-tier battery cages for 26 weeks, including a two-week pre-laying period. All birds were fed iso-nitrogenous and iso-caloric diets, and had free access to water. Control group (C) hens were fed a basal diet, and experimental group (BS) hens received a basal diet supplemented with a commercial probiotic preparation of *Bacillus subtilis* ATCC PTA-6737 at 1×10^8 CFU/kg feed. The number and weight of eggs laid, feed intake, feed conversion, egg quality, the fatty acid profile and cholesterol content of yolk lipids, and selected blood biochemical parameters of hens were determined throughout the experiment. No significant differences (P>0.05) were noted between the groups in average egg weight, laying performance (%), daily feed intake or feed conversion. Eggs laid by BS group hens received significantly higher scores for yolk color (Roche yolk color fan) and albumen quality (Haugh units), and they were characterized by a significant improvement in shell thickness and breaking strength (P<0.05) in comparison with eggs laid by control group hens. No significant differences (P>0.05) were observed between the groups in the fatty acid profile of yolk lipids, except for a significant increase (P<0.05) in oleic acid concentrations in group BS. No significant differences (P>0.05) were noted between the groups in selected blood biochemical parameters of laying hens. Group BS eggs had a significantly lower (P<0.05) cholesterol content of yolk lipids. It can be concluded that a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 had a beneficial influence on selected performance parameters of laying hens, egg quality and the cholesterol content of yolk lipids.

Key words: laying hens, *Bacillus subtilis*, performance, egg quality

The term "probiotic" comes from Greek where it means "for life" ("pro bios"). According to the definition proposed in 2002 by FAO/WHO experts, probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Gut microbiota stimulate the mucosal immune system, help to maintain intestinal homeostasis, and play an important

role in digestion and absorption (O'Hara and Shanahan, 2007; Dankowiakowska et al., 2013). Probiotics compete with pathogenic bacteria for binding sites and nutrients, thus supporting a healthy gut microbial ecosystem (Mizak et al., 2012). In poultry nutrition, priobiotic bacteria – which could offer an effective alternative to antibiotics – are expected to improve the health status of birds, enhance their disease resistance and increase productivity (Ehrmann et al., 2002; Patterson and Burkholder, 2003; Janocha et al., 2010; Deng et al., 2012). The most commonly used probiotics include bacteria of the genera *Lactobacillus, Bifidobacterium, Streptococcus, Enterococcus, Pediococcus, Lactococcus, Leuconostoc* and *Bacillus,* yeasts of the genera *Saccharomyces* and *Kluyveromyces* and fungi of the genus *Aspergillus* (Gaggia et al., 2010).

Numerous studies have concluded that probiotics used as feed additives exert a beneficial influence on egg production and the physiological parameters of birds. Probiotic bacteria affect the composition and activity of cecal microflora (Willis and Reid, 2008; Vila et al., 2009). Probiotics contained in laying hen diets contribute to improving egg quality, increasing laying rates and reducing feed costs (Panda et al., 2003; Kurtoglu et al., 2004; Panda et al., 2008; Youssef et al., 2013; Chung et al., 2015). Probiotic bacteria also help to decrease the serum cholesterol levels of layers and the cholesterol content of egg yolk (Mahdavi et al., 2005; Panda et al., 2008; Mikulski et al., 2012). The addition of probiotic bacteria to feed may have a beneficial effect on bone quality in hens (Abdelqader et al., 2013 b; Świątkiewicz et al., 2014). However, according to other authors, dietary supplementation with probiotic bacteria has no significant effect on laying performance or average egg weight (Horniakova et al., 2006; Mutus et al., 2006; Mateova et al., 2009; Zarei et al., 2011; Afsari et al., 2014).

Research into bacteria of the family *Bacillus* has demonstrated that they can effectively improve the health status and productivity of birds (Shivaramaiah et al., 2011). Various strains of *Bacillus subtilis* have been found to positively affect the health and well-being of birds. According to La Ragione et al. (2001), *Bacillus subtilis* spores can inhibit intestinal colonization by *Escherichia coli* in chickens. A similar trend was observed by La Ragione and Woodward (2003) who investigated the effect of *Bacillus subtilis* spores on *Salmonella enterica* and *Clostridium perfringens*. *Bacillus subtilis* has also been found to improve the health status of layers exposed to aflatoxin B_1 (Ma et al., 2012). Dried *Bacillus subtilis* cultures increased productivity and feed efficiency in laying hens, and decreased the cholesterol content of egg yolk (Xu et al., 2006). Ribeiro Jr. et al. (2014) observed that supplementation of layer diets with *Bacillus subtilis* improves the egg production and reduces excreta moisture. In a study of broiler chickens, the *Bacillus subtilis* PB6 strain provided health benefits and decreased the feed conversion ratio (Jayaraman et al., 2013). Thus, it appears that dietary supplementation with *Bacillus subtilis* PB6 could positively affect laying performance.

The objective of this study was to determine the effect of a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 (*Bacillus subtilis* PB6) on selected egg production parameters, egg quality, the fatty acid profile and cholesterol content of egg yolk, and selected blood biochemical parameters of laying hens.

Material and methods

Animals and dietary treatments

The experiment was conducted at the experimental poultry farm of the Department of Poultry Science, University of Warmia and Mazury (Olsztyn, Poland). All procedures used in this experiment were approved by the Animal Ethics Committee (decision No. 97/2012).

The experimental materials comprised 288 Lohmann Brown laying hens aged 16 weeks, raised in accordance with the breeder's recommendations (LTZ, 2014). The hens were reared for 26 weeks, including a two-week pre-laying period (the experiment started when 20% of the hens were in lay). At 18 weeks of age, the hens were randomly assigned to two dietary treatments (9 replicates of 16 birds each) and were kept in pairs in Big Dutchman double-sided three-tier battery cages (40 × 35 × 60 cm). The birds were housed in an environmentally controlled house with a day length of 15 h light:9 h dark.

Table 1. Composition and calculated nutrient content of experimental diets for laying hens (%)

Item	Diet	
	pre-layer	layer
Wheat	55.96	53.46
Soybean meal	21.00	21.00
Corn	11.40	-
Limestone	4.30	9.10
Rye	-	7.00
Sunflower meal	4.00	4.00
Soybean oil	1.00	3.50
MCP	1.50	1.10
Salt	0.30	0.30
$NaHCO_3$	0.15	0.15
DL-Methionine	0.14	0.14
Vit. + min. premix[1]	0.25	0.25
Calculated nutrient content[2] (g/kg)		
ME (MJ/kg)	11.51	11.49
Crude protein	184.2/180.63	179.5/178.73
Lysine	8.6	8.5
Methionine	4.3	4.1
Methionine + Cysteine	7.4	7.3
Threonine	6.5	5.1
Tryptophan	2.2	1.9
Calcium	20.3	38.3
Total phosphorus	7.3	6.3
Available phosphorus	4.4	3.6
Sodium	1.7	1.7

[1]supplied the following per kilogram of feed: 8,000 IU of vitamin A, 2,500 IU of vitamin D_3, 20 mg of vitamin E, 1 mg of vitamin K_3, 1.5 mg of vitamin B_1, 4 mg of vitamin B_2, 20 mg of vitamin B_3, 1 mg of vitamin B_6, 0.02 mg of vitamin B_{12}, 0.5 mg of folic acid, 0.1 mg of biotin, 6 mg of pantothenic acid, 45 mg of iron, 52 mg of zinc, 65 mg of manganese, 6 mg of copper, 0.7 mg of iodine, 0.15 mg of selenium.

[2]calculated (Smulikowska and Rutkowski, 2005).

[3]analyzed (Naumann and Bassler, 1993).

The diets, offered *ad libitum* in meal form, were formulated so as to meet the nutrient and energy requirements of laying hens (Smulikowska and Rutkowski, 2005). Control group (C) hens were fed a basal diet based on wheat and soybean meal, with no feed additives. Experimental group (BS) hens received a basal diet supplemented with the commercial probiotic preparation CLOSTAT containing *Bacillus subtilis* ATCC PTA-6737 (Kemin Europa NV, Belgium), at 1×10^8 CFU/kg feed. Diet composition is given in Table 1. The counts of *Bacillus subtilis* in diets C and BS fed during the laying period were also determined.

Production parameters and egg quality

The birds were weighed at the beginning (18 weeks of age) and at the end (42 weeks of age) of the trial. Eggs were collected from each cage six times per week to determine the total number of eggs laid. The average weight of eggs laid was determined by weighing eggs from each cage every week. Feed intake and feed conversion were recorded at four-week intervals.

Egg quality was analyzed at four-week intervals (week 4, 8, 12, 16, 20 and 24 of the laying period). Freshly laid eggs were collected on the first day of a given period. Each time, egg quality was evaluated on 15 eggs per group selected randomly based on the average egg weight for a given experimental period. Eggshell thickness and breaking strength, yolk color, albumen quality (Haugh unit score) and the percentage composition of egg components were determined.

Total egg weight, yolk weight and eggshell weight (including shell membranes) were determined individually on a Radwag PS1000/C/2 precision balance, with an accuracy of ± 0.01 g. The yolk was separated from the albumen using a Teflon spoon, it was rolled on a blotting paper towel to remove adhering albumen, and weighed on a precision balance. Eggshells were dried at room temperature, and weighed on a precision balance. Albumen weight was calculated as the difference between total egg weight and yolk weight + shell weight. The percentages of albumen, yolk and shell were calculated relative to total egg weight. Yolk color and Haugh unit score were determined with the use of an egg analyzer (Sanovo Engineering). Eggshell thickness was the mean value of measurements at three locations on the egg (air cell, equator and sharp end), determined using the Mitutoyo micrometer with an accuracy of ± 0.001 mm. Eggshell breaking strength was measured with the use of an egg force reader (Sanovo Engineering).

The FA composition and cholesterol content of egg yolk

At 42 weeks of age, i.e. in week 24 of the laying period, 10 eggs were picked randomly from each group to determine the fatty acid profile and cholesterol content of yolk. Fat was extracted from egg yolks with a 2:1 chloroform-methanol mixture, as described by Folch et al. (1957). The extracted fat was esterified with a chloroform-methanol-sulfuric acid mixture, as described by Peisker (Żeglarska et al., 1991). 50–60 mg of fat was transferred to Eppendorf tubes and combined with 1.5 cm^3 of a methanol-chloroform concentrated sulfuric acid mixture (100:100:1 v/v). After the fat had melted, the contents of the test tubes were transferred quantitatively to ampoules, which were sealed by melting their tops with an open flame. Sealed vi-

als were immersed in a boiling water bath for 90 min. The contents of the ampoules were shaken every 20 min.

After saponification, fatty acid methyl esters (FAMEs) were separated on an Agilent 7890A gas chromatograph (Agilent Technologies) with a flame ionization detector (FID). A Supelcowax 10 capillary column was used (length – 30 m, internal diameter – 0.32 mm, film thickness – 0.25 μm); carrier gas – helium, flow rate – 1.5 ml/min; temperature: column – 195°C, detector – 250°C, injector – 230°C. The peaks of fatty acids were identified by comparing their relative retention times with those of FAME reference standards.

Cholesterol concentrations were determined based on IDF Standard (1992) by the modified method of the International Dairy Federation (1992). 0.5 g samples of fat extracted according to the method of Folch et al. (1957) were placed in flasks and combined with 1 cm^3 of internal standard (dotriacontane $C_{32}H_{66}$) and 50 cm^3 of 2 M KOH (potassium hydroxide) in methanol. Acylglycerols were saponified in a water bath for approx. 1 hour. After saponification, the samples were cooled to 35°C, extracted three times with diethyl ether (50 cm^3 distilled water and 25 cm^3 diethyl ether) in a separatory funnel, and vigorously shaken for 1 min. The aqueous layer was transferred to another separatory funnel, and the procedure was repeated three times. Ether extracts were combined and rinsed three times with 50 cm^3 distilled water, and filtered through filter paper with ca. 25 g of anhydrous sodium sulfate. The ether was distilled off in a rotary evaporator, and the remains were dissolved in a hexane-isopropanol mixture (93:7). Separation was carried out by high-performance liquid chromatography (HPLC), as described by Nogueira and Bragagnolo (2002), on a PU-4600 (Pye Unican, Cambridge, UK) chromatograph with a flame ionization detector (FID), under the following conditions: length of a glass column – 1 m, internal diameter – 4 mm; Chromosorb W (HP) 80/100 mesh, liquid phase – OV-17; carrier gas – argon, flow rate – 50 ml/min; temperature: detector – 300°C; injector – 290°C; column – 260°C. The cholesterol content of yolk was expressed as milligrams per gram of yolk lipids.

Blood analyses

In week 24 of the laying period, blood samples were collected from the wing vein (cutaneous ulnar vein) of 8 birds from each group. The serum levels of calcium, phosphorus, triacylglycerols and cholesterol were determined with the use of the VetTest 8008 analyzer.

Statistical analysis

The results of the experiment were verified by one-way analysis of variance (ANOVA) using STATISTICA 10.0 PL software. The significance of differences between groups was determined by *F*-test. The differences were considered significant at $P \leq 0.05$.

Results

In accordance with the methodological assumptions, the concentrations of *Bacillus subtilis* ATCC PTA-6737 in diets C and BS reached 0 and 1.4×10^8 CFU per kg feed, respectively.

The body weights and weight gains of laying hens are shown in Table 2. *Bacillus subtilis* ATCC PTA-6737 added to feed had a significant effect on the final body weights (2.6%) and weight gains (12.8%) of hens. At the beginning of the experiment, the average body weight of birds was 1600 g, and in week 24 of the laying period it was consistent with the standards for Lohmann Brown hens (LTZ, 2014).

Table 2. Body weight and weight gain of laying hens (kg)

Age (weeks)	Group		SEM	P
	C	BS		
18	1.595±0.041	1.599±0.041	0.009	0.822
42	1.954±0.044 b	2.004±0.050 a	0.012	0.035
Body weight gain, 18 to 42	0.359±0.038 b	0.405±0.037 a	0.010	0.019

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

No significant differences (P>0.05) were noted between the groups in average egg weight, laying rate (%), daily feed intake or feed conversion (Table 3). The values of all parameters remained within the laying performance ranges for Lohmann Brown hens (LTZ, 2014).

Table 3. Production parameters of laying hens

Item	Group		SEM	P
	C	BS		
Egg weight (g)	61.3±0.7	61.8±1.3	0.242	0.346
Egg mass (g/hen)	56.5±1.2	57.5±1.2	0.282	0.419
Laying rate (%)	92.2±1.2	92.2±1.0	0.250	0.963
Feed intake (g/hen)	118.1±1.8	119.1±2.3	0.483	0.337
FCR (g feed/g egg mass)	2.19±0.10	2.18±0.09	0.017	0.919

The physical properties of eggs are presented in Table 4. Dietary supplementation with *Bacillus subtilis* PB6 contributed to a significant increase (P<0.05) in shell thickness and breaking strength, and shell weight as a percentage of total egg weight. The yolks of eggs laid by BS group hens had a darker color (15.1%). An improvement in albumen quality (Haugh units) was also noted in group BS (3.5%). The probiotic preparation had no influence (P>0.05) on the percentages of yolk and albumen in total egg weight.

Table 5 presents the effect of dietary *Bacillus subtilis* PB6 on the fatty acid profile of yolk lipids in laying hens at 42 weeks of age. The predominant fatty acids were oleic acid, palmitic acid, linoleic acid and stearic acid. No significant differences (P>0.05) were observed between the groups in the fatty acid profile of yolk lipids, except for a significant increase (P<0.05) in oleic acid (cis11) concentrations in group BS. The concentrations of saturated and unsaturated fatty acids were similar in

both groups. Dietary *Bacillus subtilis* PB6 had no significant effect on the nutritional value of eggs.

Table 4. Effect of dietary *Bacillus subtilis* ATCC PTA-6737 on egg quality

Item	Group		SEM	P
	C	BS		
Shell thickness (mm)	0.355±0.008 b	0.365±0.008 a	0.002	0.007
Shell strength (N)	45.12±2.30 b	47.63±2.78 a	0.572	0.025
Yolk color (points)	7.83±0.83 b	9.01±0.71 a	0.194	0.001
Haugh units	70.45±3.45 b	72.95±2.59 a	0.656	0.043
Egg composition (%)				
yolk	23.78±0.61	23.54±0.40	0.106	0.244
albumen	66.43±0.68	66.42±0.44	0.114	0.992
shell	9.79±0.18 b	10.04±0.15 a	0.043	0.001

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

Table 5. Effect of dietary *Bacillus subtilis* ATCC PTA-6737 on the fatty acid profile (% of total fatty acid content) of egg yolk in laying hens at 42 weeks of age

Item	Group		SEM	P
	C	BS		
Myristic acid (C14:0)	0.35±0.03	0.37±0.04	0.009	0.193
Myristoleic acid (C14:1)	0.12±0.18	0.07±0.01	0.031	0.393
Pentadecanoic acid (C15:0)	0.07±0.01	0.07±0.01	0.002	0.967
Palmitic acid (C16:0)	23.93±0.55	24.35±0.94	0.195	0.298
Palmitoleic acid (C16:1)	3.03±0.37	2.93±0.47	0.103	0.631
Margaric acid (C17:0)	0.20±0.02	0.21±0.03	0.007	0.594
Margaroleic acid (C17:1)	0.17±0.01	0.17±0.03	0.005	0.969
Stearic acid (C18:0)	8.10±0.33	7.84±1.15	0.206	0.547
Oleic acid (C18:1) cis9	38.81±1.66	38.93±2.35	0.492	0.912
Oleic acid (C18:1) cis11	1.78±0.12 b	1.93±0.15 a	0.039	0.039
Linoleic acid (C18:2*n-6*)	18.90±1.30	18.86±1.55	0.345	0.955
Linolenic acid (C18:3*n-3*)	1.30±0.12	1.29±0.16	0.034	0.901
Eicosenoic acid (C20:1)	0.17±0.01	0.17±0.02	0.004	0.878
Eicosadienoic acid (C20:2)	ND	ND	-	-
Arachidonic acid (C20:4*n-6*)	1.86±0.15	1.74±0.50	0.091	0.531
Docosahexaenoic acid (C22:6*n-3*)	1.20±0.10	1.06±0.32	0.060	0.283
SFAs	32.66±0.65	32.85±1.63	0.300	0.763
MUFAs	44.08±1.69	44.20±2.78	0.556	0.924
PUFAs	23.26±1.46	22.95±2.04	0.430	0.739
UFAs	67.34±0.65	67.15±1.63	0.300	0.763
n-6 PUFAs	20.76±1.33	20.60±1.72	0.371	0.838
n-3 PUFAs	2.50±0.18	2.35±0.34	0.068	0.307
n-6/n-3 PUFA ratio	8.32±0.40	8.83±0.74	0.157	0.110

a, b – values in rows with different letters differ significantly (P<0.05).
ND – not detected.
SFAs – saturated fatty acids.
MUFAs – monounsaturated fatty acids.
PUFAs – polyunsaturated fatty acids.

Table 6 shows the cholesterol content of yolk lipids in laying hens at 42 weeks of age and selected blood biochemical parameters. The cholesterol content of yolk lipids was significantly lower (P<0.05) in group BS, whereas blood cholesterol levels were comparable (P>0.05) in both groups. The probiotic preparation containing *Bacillus subtilis* PB6 had no influence (P>0.05) on the serum levels of calcium, phosphorus and triacylglycerols.

Table 6. Effect of dietary *Bacillus subtilis* ATCC PTA-6737 on selected blood biochemical parameters and yolk cholesterol content in laying hens at 42 weeks of age

Item	Group		SEM	P
	C	BS		
Content in blood (mg/dl)				
Ca	24.1±5.2	25.6±1.7	0.954	0.466
P	5.8±1.2	6.4±1.0	0.286	0.243
triacylglycerols	1760.4±775.7	1856.0±691.1	177.9	0.798
cholesterol	112.9±24.5	100.4±26.9	6.419	0.348
Content in egg yolk fat (mg/g)				
cholesterol	28.1±2.0 b	24.8±4.6 a	0.689	0.013

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

Discussion

The results of our study indicate that dietary *Bacillus subtilis* ATCC PTA-6737 led to a significant increase in the final body weights and weight gains of laying hens. Panda et al. (2003, 2008) observed no differences in the weight gains of hens fed diets supplemented with probiotic bacteria. Gallazzi et al. (2008) demonstrated that dietary supplementation with *Lactobacillus acidophilus* had no influence on the weight gains of layers. In a study by Abdelqader et al. (2013 a), the body weights of laying hens were not affected by feed additives such as *Bacillus subtilis*, inulin or synbiotics. The increase in the final body weights and weight gains of laying hens, noted in the present study, may be due to the development of beneficial bacteria in the digestive tract of layers, which could improve their performance (Edens, 2003). Improvement of the intestinal environment may contribute to increasing the efficiency of nutrient digestion and absorption (Pelicano et al., 2004).

In our experiment, a probiotic preparation containing *Bacillus subtilis* PB6 had no significant effect on production parameters such as egg weight, laying rate, feed intake or feed conversion. Gallazzi et al. (2008) did not note any improvements in laying performance, egg weight, feed intake or feed efficiency in hens fed diets supplemented with *Lactobacillus acidophilus*. Other studies also revealed that probiotics had no influence on performance parameters such as egg weight and feed conversion (Panda et al., 2003), laying rate, egg weight and feed intake (Panda et al., 2008), egg weight, feed intake and feed efficiency (Quarantelli et al., 2008), laying rate and egg weight (Balevi et al., 2009), laying rate, feed intake and feed conversion (Ramasamy et al., 2009), or laying rate and feed intake (Mikulski et al., 2012).

Abdelqader et al. (2013 a, b) reported higher laying rates, higher egg weight and a lower feed conversion ratio in groups receiving *Bacillus subtilis*.

Bacillus subtilis ATCC PTA-6737 added to feed for laying hens contributed to an improvement in eggshell quality. Li et al. (2006) found that dried *Bacillus subtilis* cultures increased eggshell thickness. Panda et al. (2008) also demonstrated that shell quality parameters improved in response to the dietary inclusion of probiotic bacteria (*Lactobacillus sporogenes*). Abdelqader et al. (2013 a, b) reported an increase in eggshell thickness and shell weight as a percentage of total egg weight in laying hens fed dietary *Bacillus subtilis*. According to Aghaei et al. (2010), Mikulski et al. (2012) and Youssef et al. (2013), probiotics exerted a beneficial influence on eggshell thickness. This beneficial effect may be attributed to a favorable environment in the gastrointestinal tract resulting from the administration of probiotics to birds (Mohan et al., 1995; Panda et al., 2008; Mikulski et al., 2012). Probiotic bacteria increase the rate of fermentation and the production of short-chain fatty acids (SCFAs), which reduces the luminal pH (Scholz-Ahrens et al., 2007). Low luminal pH increases calcium solubility and absorption (Van den Heuvel et al., 1999). SCFAs stimulate intestinal epithelial cell proliferation and villus height (Garcia et al., 2007), which increases absorption efficiency (Scholz-Ahrens et al., 2007). As a result, more nutrients, including calcium, can be assimilated, thus improving eggshell quality.

In our study, eggs from hens fed diets containing *Bacillus subtilis* received significantly higher scores for yolk color and albumen quality (Haugh units). A darker color of yolk in eggs from laying hens receiving probiotic bacteria was also reported by other authors (Li et al., 2006; Mikulski et al., 2012; Youssef et al., 2013). However, Xu et al. (2006) and Zhang et al. (2012) did not note any changes in yolk color in response to probiotic bacteria. In a study by Zhang et al. (2012) and Chung et al. (2015) probiotic bacteria had a positive effect on albumen quality, whereas such an influence was not observed in other experiments (Mahdavi et al., 2005; Xu et al., 2006; Gallazzi et al., 2008; Panda et al., 2008; Mikulski et al., 2012; Youssef et al., 2013). According to Mahdavi et al. (2005), there is no reasonable explanation for the improvement in albumen quality in the microbial additive group. It might be speculated that carotenoids from the diet can be well absorbed and transferred into the egg yolk, thus improving yolk color. Carotenoid-rich diets are associated with lower serum cholesterol concentrations (Yeum and Russell, 2002).

In the present study, *Bacillus subtilis* ATCC PTA-6737 had no significant effect on the fatty acid profile of yolk lipids, except for a significant increase in oleic acid concentrations. Haddadin et al. (1996) and Ramasamy et al. (2009) also demonstrated that the inclusion of probiotics in laying hen diets had no significant influence on the fatty acid profile of egg yolk. Mikulski et al. (2012) reported that *Pediococcus acidilactici* bacteria increased the concentrations of linoleic acid, linolenic acid and PUFAs in the egg yolk.

In our experiment, *Bacillus subtilis* PB6 had no significant effect on selected blood biochemical parameters of hens, but contributed to a significant decrease in the cholesterol content of egg yolk. Mateova et al. (2009) also demonstrated that probiotic bacteria had no influence on the blood biochemical parameters of laying hens. According to Panda et al. (2003), probiotics increased blood calcium levels and

decreased blood cholesterol levels and the cholesterol content of yolk lipids. Capcarova et al. (2010) reported that the blood biochemical parameters of laying hens were affected by the *Enterococcus faecium* M74 strain. The results of numerous studies indicate that probiotic bacteria reduce cholesterol concentrations in yolk lipids (Mahdavi et al., 2005; Xu et al., 2006; Panda et al., 2008; Ramasamy et al., 2009; Mikulski et al., 2012). Nelson and Gilliland (1984) and Gilliland et al. (1985) suggested that certain microorganisms present in probiotics may assimilate cholesterol from the gastrointestinal tract for their metabolism, thus reducing the amount of cholesterol absorbed. According to Fukushima and Nakano (1995), probiotics are able to inhibit the activity of hydroxymethyl-glutaryl-coenzyme A in the gastrointestinal tract and in this way decrease cholesterol levels. Probiotic bacterial strains are also able to modify the enterohepatic cycle and reduce cholesterol through assimilating dietary cholesterol into bacterial cells and deconjugation of bile salts in the intestine, thus preventing them from acting as precursors in cholesterol synthesis (Abdulrahim et al., 1996; St-Onge et al., 2000; Kalavathy et al., 2003).

It can be concluded that a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 had a beneficial influence on selected performance parameters of laying hens, egg quality and the cholesterol content of yolk lipids.

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