Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 57 (2), 2018: 95–101
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 57 (2), 2018)
DOI 10.22630/AAS.2018.57.2.9

Effect of *Bacillus subtilis* and *Bacillus licheniformis* inclusion in turkey diets on growth performance

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Abstract: *Effect of* Bacillus subtilis *and* Bacillus licheniformis *inclusion in turkey diets on growth performance.* The aim of this study was to evaluate the influence of a probiotic preparation containing spores of *Bacillus subtilis* and *Bacillus licheniformis*, added to feed, on growth performance of female turkeys reared until 84th day of age. A total of 300-day-old Hybrid Converter female turkeys were randomly assigned to 20 pens. The pens were randomly divided into two treatment groups: T1 received basal diets, and T2 received the same diets as group T1 supplemented with the probiotic ($1.28 \cdot 10^6$ CFU/g feed). It can be concluded that the probiotic feed additive had a beneficial influence on growth performance and significantly increased the final body weights and average daily gains of female turkeys.

Key words: female turkeys, probiotic, *Bacillus subtilis*, *Bacillus licheniformis*, growth performance

INTRODUCTION

Poultry production has been growing rapidly in recent years due to the fast growth rate and short fattening period of birds. This prompted the search for new solutions in poultry nutrition, to improve productivity while maintaining the desirable taste of meat as well as adequate standards of poultry health and welfare. Feed additives, including probiotics, are increasingly used in poultry nutrition on account of their positive effects on gut microbiota (Fallah et al. 2013). An improvement in the intestinal environment may contribute to increasing the efficiency of nutrient digestion and absorption (Pelicano et al. 2004). Probiotic strains produce bacteriocins with bacteriostatic activity (Oelschlaeger 2010). Dietary supplementation with probiotics exerts positive effects on local (GALT) and humoral immunity in poultry (Alloui et al. 2013). Probiotic bacteria produce their own enzymes and activate the host's enzymes, thus improving the growth rate of birds and feed conversion ratio (Yirga 2015). *Bacillus subtilis* and *Bacillus licheniformis* strains are widely used in probiotic products (Hong et al. 2005). The aim of this study was to evaluate the influence of a probiotic preparation containing spores of *B. subtilis* and *B. licheniformis*, added to feed, on the growth performance of female turkeys reared until 84th day of age.

MATERIAL AND METHODS

The experiment was conducted at the experimental poultry farm of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn (Poland). There were two dietary treatments in the experiment: T1 – control treatment without dietary supplementation, and T2 – experimental treatment with dietary supplementation with a probiotic. The test material (BioPlus 2B, Chr.Hansen A/S, Denmark) contained strains of *B. subtilis* and *B. licheniformis* at a ratio of 1 : 1 ($1.6 \cdot 10^9$ *B. subtilis* spores and $1.6 \cdot 10^9$ *B. licheniformis* spores per 1 g of the preparation). *Bacillus subtilis* and *B. licheniformis* were added to T2 diets at $1.28 \cdot 10^6$ CFU/g feed (400 g/t feed). A total of 300-day-old healthy female turkey poults (Hybrid Converter) were obtained from a commercial hatchery. The turkeys were allocated at random to 20 floor pens. Each treatment comprised 10 pens (replicates) of 15 birds each. Pen surface area was 4 m^2 (stocking density of 3.75 birds per 1 m^2). Wood shavings were used as bedding material, and the litter was replenished as necessary. The house was provided with artificial programmable lights and climate, heating by gas heating system, and forced ventilation. The environmental conditions were consistent with the Hybrid recommendations. The trial was conducted for 84 days, and involved three feeding phases. The diets were formulated and the calculations were performed based on the dietary recommendations for female turkeys proposed by Smulikowska and Rutkowski (2005). Each pen was equipped with a feeder, and feed was offered *ad libitum*. Feeders were re-filled with pre-weight feed amounts when required. All diets were offered in crumbled/pelleted form. Drinking water was supplied *ad libitum* by bell-type drinkers. All experimental procedures involving animals were approved by the local Animal Experimentation Ethics Committee at the University of Warmia and Mazury in Olsztyn.

The birds were weighed on the day of arrival, and then the body weight (BW) of birds in each pen was recorded on a pen basis at 28th, 56th and 84th day of age. Feed intake was calculated as the difference between the offered feed and refusals. The results were used to determine feed conversion ration (FCR) for all experimental periods, and the European productivity index – EPI [(livability × × final BW × 100)/(duration of the study × × FCR)] for the entire experiment. All diets were analyzed for the content of crude nutrients by the VDLUFA method (Naumann and Bassler 1993), and for the content of *Bacillus* spores.

The results were analyzed by a one-way analysis of variance (ANOVA), and significant differences between treatments were determined by Duncan's multiple range test. The Statistica software package ver. 10.0 was used for statistical calculations. The data in tables are given as means and standard deviations. Treatment differences were considered significant at $P \leq 0.05$. Replicate-pen was the experimental unit for all variables measured.

RESULTS AND DISCUSSION

A proximate feed analysis showed that the actual nutrient concentrations were consistent with the calculated values (Table 1). The mean concentrations of

TABLE 1. Composition and nutrient density of the diets

Composition (%)	Feeding phase		
	Starter (1–28 days)	Grower (29–56 days)	Finisher (57–84 days)
Wheat	42.711	46.417	53.805
Sunflower meal	3.000	4.000	5.000
Soybean meal	40.172	37.411	27.804
Rapeseeds	3.000	4.000	5.000
Potato protein	3.000	–	–
Soybean oil	2.928	2.760	2.477
Animal fat	–	1.000	2.000
Na-bicarbonate	0.100	0.100	0.100
Salt	0.229	0.207	0.214
Limestone	1.824	1.518	1.524
MCP	1.898	1.419	0.978
Choline chloride	0.070	0.070	0.070
DL-methionine	0.313	0.303	0.268
L-lysine	0.372	0.438	0.384
L-threonine	0.134	0.107	0.126
Vitamins + trace minerals[1, 2]	0.250	0.250	0.250
Nutrient density[3]			
Age (weeks)	0–4	5–8	9–12
ME (kcal/kg)	2 800	2 880	3 000
Crude protein (g/kg)	280.0/277.2[4]	255.0/248.7[4]	225.0/221.1[4]
Methionine (g/kg)	7.2	6.7	6.00
Methionine + Cysteine (g/kg)	11.8	11.0	10.0
Lysine (g/kg)	17.5	16.0	13.5
Threonine (g/kg)	11.6	10.0	9.0
Arginine (g/kg)	17.7	16.3	14.2
Calcium (g/kg)	12.0	10.0	9.0
Phosphorus (g/kg)	6.0	5.0	4.0

[1] Content per kg premix for weeks 1–8: 5,000,000 IU Vitamin A; 1,330,000 IU Vitamin D_3; 670,000 IU Vitamin D_3 HyD; 40,000 mg Vitamin E; 1,600 mg Vitamin K_3; 1,800 mg Vitamin B_1; 6,000 mg Vitamin B_2; 2,000 mg Vitamin B_6; 16 mg Vitamin B_{12}; 1,400 mg Folic acid; 11,200 mg Pantotenic acid; 44,000 mg Nicotinic acid; 150 mg Biotin; 64,000 mg Manganese; 64,000 mg Zinc; 32,000 mg Iron; 10,000 mg Coper; 1,000 mg Iodine; 120 mg Selenium.

[2] Content per kg premix for weeks 9–12: 3,840,000 IU Vitamin A; 1,920,000 IU Vitamin D_3; 24,000 mg Vitamin E; 1,200 mg Vitamin K_3; 800 mg Vitamin B_1; 4,800 mg Vitamin B_2; 2,000 mg Vitamin B_6; 10 mg Vitamin B_{12}; 1,000 mg Folic acid; 9,200 mg Pantotenic acid; 34,000 mg Nicotinic acid; 150 mg Biotin; 48,000 mg Manganese; 48,000 mg Zinc; 16,000 mg Iron; 10,000 mg Coper; 800 mg Iodine; 120 mg Selenium.

[3] Calculated (Smulikowska and Rutkowski 2005).

[4] Analyzed (Naumann and Bassler 2004).

B. subtilis and *B. licheniformis* in turkey diets were $9.35 \cdot 10^5$, $9.23 \cdot 10^5$ and $1.26 \cdot 10^6$ viable cells per 1 g of feed in the starter, grower and finisher phases, respectively. The probiotic strains were not detectable in control diets (< $1.0 \cdot 10^4$ CFU/g feed). The results were satisfactory and corresponded to the target values, 0 and $1.28 \cdot 10^6$ CFU/kg feed in groups T1 and T2, respectively. The livability was very good in both treatments – 100%. During the experiment, 22 birds (6.67 and 8% in T1 and T2, respectively) were culled. The main reason for culling was enlarged crop (20 birds), and it was no relationship between the reason of culling and the use of the probiotic preparation.

During the first feeding phase, the tested probiotic had no significant effect of feed intake in female turkeys fed crumbled/pelleted diets (Table 2). Differences in feed intake were noted in the period of 1–56 days when birds fed probiotic-supplemented diets consumed more feed than control group birds ($P = 0.048$). Over the entire experimental period of 84 days the female turkeys from the probiotic group (T2) consumed 2.7% more feed than the control group (T1) birds. The difference in feed intake (1–84 days) was not significant ($P = 0.230$), but it could suggest that probiotic bacteria (*B. subtilis* and *B. licheniformis*) exerted a stimulatory effect. An increase in feed intake due to dietary probiotic supplementation has been found to improve the growth performance of birds. However, in the study of Midilli et al. (2008), where the BioPlus 2B feed additive was used, no differences were observed in feed intake and consequently in the body weights or average daily gains of broiler chickens between treatments. Similar results (an improvement of growth performance) were reported by Gohain and Sapcota (1998). In contrast, Safalaoh (2006) reported improved body weight gain (BWG) and FCR in broilers supplemented with a microbial preparation (Effective Micro-organisms – EM), despite a decrease in feed intake in the experimental group.

The average body weights and weight gains of turkeys are presented in Tables 3 and 4. No significant differences in body weight gains were found between treatments in the first stage of the study (days 1–28). During the second experimental period (days 29–56), turkeys fed the BioPlus 2B diet (T2) were significantly heavier and gained significantly more than birds fed the control diet (+4.9%,

TABLE 2. The results of feed intake of female turkeys (g/day/bird)

Period (days)	Group		*SEM*	*P*
	T1	T2		
1–28	43.8 ±1.4	43.7 ±1.7	0.344	0.894
29–56	171.9 ±6.8	176.9 ±6.9	1.598	0.124
1–56	109.7^b ±3.9	113.8^a ±4.7	1.046	0.048
57–84	288.8 ±25.3	300.8 ±16.2	4.821	0.223
1–84	172.6 ±7.9	177.3 ±8.8	1.894	0.230

Values in same rows with no common superscript are significantly different ($P \leq 0.05$).

TABLE 3. The results of body weight of female turkeys (kg)

Age of birds (day)	Group		*SEM*	*P*
	T1	T2		
1st	0.057 ±0.001	0.057 ±0.001	0.000	0.280
28th	1.043 ±0.033	1.044 ±0.036	0.008	0.966
56th	3.831^{b} ±0.096	3.970^{a} ±0.124	0.029	0.012
84th	7.363^{b} ±0.243	7.659^{a} ±0.268	0.065	0.019

Values in same rows with no common superscript are significantly different ($P \leq 0.05$).

TABLE 4. The results of weight gain of female turkeys (g/day)

Period (days)	Group		*SEM*	*P*
	T1	T2		
1–28	35.2 ±1.2	35.2 ±1.3	0.268	0.981
29–56	99.6^{b} ±2.7	104.5^{a} ±3.6	0.892	0.003
1–56	67.4^{b} ±1.7	69.9^{a} ±2.2	0.516	0.012
57–84	126.1 ±8.1	131.8 ±6.4	1.711	0.102
1–84	87.0^{b} ±2.9	90.5^{a} ±3.2	0.776	0.019

Values in same rows with no common superscript are significantly different ($P \leq 0.05$).

104.5 vs. 99.6 g, $P = 0.003$). From 56th day of the trial, turkeys fed the probiotic diet (T2) were characterized by significantly higher average body weight (+3.5%, 3.831 vs. 3.970 g, $P = 0.012$) and gained significantly more in comparison with birds fed control diet (+3.7%, 69.9 vs. 67.4 g, $P = 0.012$). During the third feeding phase (days 57–84), a numerical improvement in body weight gains was noted in turkeys fed the BioPlus 2B diet (T2) relative to birds fed control diet. At 84th day, birds fed the T2 BioPlus 2B diet were heavier than birds fed the control diet (+4.02%, 7.659 vs. 7.363 kg), and the observed difference was statistically significant ($P = 0.019$). Over the entire experimental period (days 1–84), turkeys fed the BioPlus 2B diet (T2) were characterized by significantly higher body weight gains in comparison with birds fed the control diet (+4.02%). Previous studies have showed the efficacy of *B. licheniformis* and *B. subtilis* in turkey production. Fallah et al. (2013) demonstrated that a probiotic preparation containing *B. subtilis* and *B. licheniformis* had a positive influence on body weight gains in broiler chickens. In a study by Blair et al. (2004), the body weights of 18-week-old turkeys fed diets supplemented with *B. subtilis* were higher than the body weights of control group birds (14.32 vs. 13.41 kg). Shivaramaiah et al. (2011) reported significantly higher body weights in male turkeys receiving *B. subtilis*, compared with control group birds.

In the present study, dietary probiotic supplementation had no impact on FCR in any of the feeding phases and throughout the experiment (Table 5). Midilli et al.

TABLE 5. The results of feed conversion ratio (FCR) of female turkeys (kg feed/1 kg body weight gain) and European productivity index (EPI)

Period (days)	Group		*SEM*	*P*
	T1	T2		
FCR				
1–28	1.244 ±0.036	1.241 ±0.038	0.008	0.848
29–56	1.712 ±0.047	1.694 ±0.037	0.009	0.369
1–56	1.590 ±0.032	1.579 ±0.032	0.006	0.429
57–84	2.329 ±0.099	2.261 ±0.141	0.028	0.229
1–84	1.936 ±0.050	1.900 ±0.058	0.012	0.147
EPI				
1–84	453^{b} ±23	481^{a} ±28	6.451	0.028

Values in same rows with no common superscript are significantly different ($P \leq 0.05$).

(2008) noted a positive effect of dietary probiotic and prebiotic supplementation on feed intake. In an experiment with broiler chickens, Jayaraman et al. (2013) demonstrated that *B. subtilis* bacteria provided health benefits and decreased FCR.

Female turkeys from group T2, fed probiotic-supplemented diets, achieved higher productive performance throughout the experiment, and were characterized by a significantly higher EPI (+6.1%, 481 vs. 453, $P = 0.028$). Loeffler (2014) pointed out that there is evidence to support that multi-species probiotic supplementation is more efficient than single strain probiotics in turkey nutrition. In this sense, Blanch and Rouault (2016) recently concluded that the high efficiency of BioPlus 2B diet could be elucidated by the complementarity between the different modes of action of the two strains enclosed in the product, being *B. licheniformis* extremely efficient in pathogen growth inhibition and *B. subtilis* in digestive enzyme activation.

CONCLUSIONS

The results of this study indicate that a probiotic preparation containing *B. subtilis* and *B. licheniformis* positively affected the growth performance of female turkeys. Birds fed probiotic-supplemented diets were characterized by significantly higher final body weight and body weight gain, and significantly higher values of the EPI.

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Streszczenie: *Wpływ zastosowania do paszy* Bacillus subtilis *i* Bacillus licheniformis *na wyniki odchowu indyków.* Doświadczenie przeprowadzono w celu określenia wpływu zastosowania preparatu probiotycznego zawierającego spory *Bacillus subtilis* i *Bacillus licheniformis* dodanego do paszy na wyniki odchowu indyczek odchowywanych do 84. dnia życia. Trzysta jednodniowych indyczek Hybrid Converter zostało przydzielonych do 20 kojców, po 15 indyczek w każdym. Zostały one podzielone na dwie grupy (10 powtórzeń/10 kojców w każdej): T1 żywione paszą bazową oraz T2 żywione paszą bazową z dodatkiem preparatu probiotycznego w ilości $1{,}28 \cdot 10^6$ CFU/g paszy. Reasumując, dodatek probiotyku do paszy miał korzystny wpływ na wyniki odchowu indyczek i istotnie poprawiał ich końcową masę ciała oraz średnie przyrosty masy ciała.

Słowa kluczowe: indyczki, probiotyk, *Bacillus subtilis*, *Bacillus licheniformis*, wyniki odchowu

MS received 13.11.2017
MS accepted 09.05.2018

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