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Influence of a probiotic preparation on the performance of growing male turkeys

Wpływ preparatu probiotycznego na wyniki odchowu młodych indorów

Summary. The aim of the study was to determine the effect of dietary probiotic *Enterococcus faecium,* DSM 10663 NCIMB 10415, on the performance of growing male turkeys in a 105-day trial, in a 5-phase feeding. A total of 450 male turkey poults (BUT Big 6 hybrids) were randomly divided into two treatment groups (9 replicates of 25 birds each). All birds were fed isonitrogenous and isoenergetic diets, consisting of a control group (C) – basal diet, and an experimental group (EF) – basal diet supplemented with *Enterococcus faecium* at 1.05 x 10^9 cfu kg^{-1} feed (30 g t^{-1} feed). Performance parameters, including body weight, feed intake and feed conversion ratio, were determined. The addition of *Enterococcus faecium* preparation to the feed mixture (group EF) significantly increased ($p \leq 0.05$) the body weight of turkeys on the 77^{th} and 105^{th} days of age, respectively about 4.4 and 3.3% more than the control group – C. The same tendency was also observed in the body weight gain. The results of this experiment are in accordance with other studies on this subject regarding the use of probiotics and their beneficial effect on the performance. Probiotics are an alternative to the now-banned feed antibiotics in the EU. The use of *Enterococcus faecium,* DSM 10663 NCIMB 10415, can be a performance stabilizing factor in the relatively long fattening period of turkeys.

Key words: turkeys, probiotic, *Enterococcus faecium,* performance

INTRODUCTION

The total prohibition of the usage of antibiotic growth promoters in poultry feeding since 2006 is creating a need for alternative feed supplements like enzymes, probiotic, organic acids, as well as various prebiotic and phytogenic preparations, which stimulate growth of a beneficial microflora in the alimentary tract. One of these alternatives are probiotics [Simon and Flachowsky 2006]. Probiotics are live bacteria or yeast preparations containing microorganisms of one or several kinds, which have an antagonistic effect on pathogenic bacteria in intestines as well as a positive effect on microflora in intestines and the macro organism itself

[Vanbella *et al*. 1990]. With turkeys, growth trials with a focus on the efficiency of supplementary probiotics have rarely been conducted. Therefore, and because of the request for approval, an experiment was conducted to study the efficiency of probiotic preparations (with *Enterococcus faecium*, DSM 10663 NCIMB 10415) in growing male turkeys.

MATERIALS AND METHODS

There were two treatments in the experiment, treatment 1 (C) – a control treatment without any addition to the feed, and treatment 2 (EF) with probiotic supplementation to the diet. The *Enterococcus faecium*, DSM 10663 NCIMB 1041, supplementation to the EF diets was 1.05×10^9 cfu kg^{-1} feed (30 g t^{-1} feed). 451 day-old healthy male turkey poults (BUT Big 6) hybrids were obtained from a commercial hatchery. The turkeys were allocated at random to 18 pens, measuring 10.0 m^2 each. Each treatment comprised 9 floor pens (replicates), with 25 birds in one pen per replicate. The pen surface was 10 m^2 (stocking density 2.5 birds per m^2). Straws were used as bedding material and additional straw was added when necessary. The house was provided with artificial programmable lights and climate and was heated by a gas heating system with forced ventilation. The environmental conditions were according to the BUT recommendations. The trial was conducted for 15 weeks and five feeding phases in different durations were used. Table 1 shows the calculated composition of the diets. The composition of the diets and the calculations were based on the recommendations given by Smulikowska and Rutkowski [2005] for male turkeys. Each pen was equipped with a feeder and the feed was offered *ad libitum*. Feeders were refilled with pre-weight amounts when required. During the first 10 days, feed was additionally offered in the flat plastic bowls. All diets used in the trial were offered in meal form. Drinking water was supplied *ad libitum* by a bell-type drinker. 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 first weighed on the day of arrival. Then, the weight of birds in each pen was recorded at the 14^{th}, 35^{th}, 63^{rd}, 77^{th} and 105^{th} day of age. Feed intake was measured by weighing the offered feed and the feed refusals.

All diets were analyzed for the content of crude nutrients by the VDLUFA method [Naumann and Bassler 1993]. The activity of *Enterococcus faecium* was measured in the Institute of Animal Nutrition (Berlin) using colony hybridization of *Enterococcus* spp. grown on Slanetz-Bartley agar (Oxoid, Basingstoke, Hampshire, UK) in combination with a strain-specific oligonucleotide probe as described [Macha *et al*. 2004].

The results of the experiment were analyzed using a one-way analysis of variance (ANOVA) and significant differences between treatments were determined with Duncan's multiple range test. The Statistica software package version 10.0 [StatSoft Inc. 2011] was used for statistical calculations. The data in tables are given as means and standard deviations.

RESULTS

Since the experiment proceeded as planned, the data from all turkeys could be included in the evaluation. A proximate feed analysis showed that the actual nutrient con-

centrations were consistent with the calculated values (Tab. 1). The mean concentration of the supplemented *E. faecium* in the feed for turkeys (EF group) was 1.21×10^9, 1.26×10^9, 1.15×10^9, 1.06×10^9 and 1.11×10^9 viable cells g^{-1} of feed, in the following feeding phases (1–2, 3–5, 6–9, 10–11 and 12–15 weeks), respectively. The probiotic strain was not detectable in the control feed. The results were satisfactory and corresponded with the target values, 0 and 1.05×10^9 cfu kg^{-1} feed in C and EF group, respectively.

Table 1. Composition and nutrient content of experimental diets for growing male turkeys
Tabela 1. Skład i zawartość składników pokarmowych w paszach doświadczalnych dla rosnących indorów

Specification Wyszczególnienie	Feeding phase (weeks) Fazy żywieniowe (tygodnie)				
	I (1–2)	II (3–5)	III (6–9)	IV (10–11)	V (12–15)
Composition/Skład (g kg^{-1})					
Corn/Kukurydza	300.0	300.0	300.0	300.0	300.0
Wheat/Pszenica	98.5	188.8	306.6	392.6	424.7
Soybean meal Poekstrakcyjna śruta sojowa	480.00	400.0	290.0	206.6	170.0
Fish meal/ Mączka rybna	50.3	28.0	20.0	15.0	10.0
Soya oil/ Olej sojowy	20.0	26.4	29.2	33.0	40.0
Limestone/ Kreda paszowa	16.3	17.0	19.3	18.0	19.0
MCP	19.2	22.6	15.9	15.9	16.7
Premix[1]/Premiks[1]	12.0	12.0	12.0	12.0	12.0
Lysine/Lizyna	1.4	2.2	3.6	2.5	3.6
Methionine/Metionina	1.5	1.9	1.5	2.5	2.0
Threonine/ Treonina	-	1.1	1.2	1.1	1.2
Choline chloride/ Chlorek choliny	0.8	0.8	0.8	0.8	0.8
Energy and nutrient content/ Energia i zawartość składników pokarmowych					
AME_N/EM_N (MJ kg^{-1})[2]	11.6	11.9	12.2	12.4	12.6
Crude protein[3]/ Białko ogólne[3]	297.4	253.7	220.6	187.9	167.5
Starch[3]/Skrobia[3]	271.2	316.2	381.0	450.4	483.2
Sugars[3]/Cukry[3]	59.3	49.8	43.7	41.3	38.1
Crude fiber[3]/ Włókno surowe[3]	41.5	42.7	39.5	35.7	31.8
Crude fat[3]/ Tłuszcz surowy[3]	44.7	49.5	51.8	57.3	63.7
Calcium[3]/ Wapń[3]	12.9	12.5	11.5	9.5	9.5
Phosphorus[3]/Fosfor[3]	10.0	9.5	8.7	7.1	7.4
Sodium[3]/Sód[3]	2.8	2.5	2.6	2.7	2.7
Lysine/Lizyna[2]	18.0	16.0	14.5	10.9	10.5
Methionine[2]/Metionina[2]	6.0	5.8	4.9	5.4	4.7
Cystine[2]/Cystyna[2]	4.4	4.0	3.6	3.3	3.0
Threonine[2]/Treonina[2]	12.1	10.2	8.6	7.9	6.3
Tryptophane[2]/Tryptofan[2]	3.3	2.9	2.4	2.1	1.8

[1]Supplied per kilogram of diet/ Suplementacja na kilogram paszy: 12000 IU Vit. A; 4800 IU Vit. D_3; 48 mg Vit. E; 3.5 mg Vit. K_3; 2.4 mg Vit. B_1; 9.4 mg Vit. B_2; 79 mg niacine; 5.8 mg Vit. B_6; 36 µg Vit. B_{12}; 240 µg biotin; 26 mg pantothenic acid; 1.8 mg folic acid; 115 mg Zn; 60 mg Fe; 102 mg Mn; 18 mg Cu; 1.4 mg Co; 0.48 mg Se; 1.9 g Na; 0.6 g Mg; 90 mg Coxidin

[2] Calculated/Wartości obliczone [Smulikowska and Rutkowski 2005]

[3] Analyzed/ Wartości analityczne [Naumann and Bassler 1993]

The experiment was performed without any problem. The survivability in both treatments was good – 96.0% and 96.9% in the group C and EF, respectively and was better compared to the BUT recommendations (95.3%). The main causes of death were obstruction of the alimentary tract by litter, intestinal enteritis (during the first feeding phase) and cachexia (remaining period) and there was therefore no relationship between the causes of death and the use of the experimental preparation.

By using meal feed mixtures to farm animals throughout the experiment, no significant effect of the tested probiotic on feed consumption was obtained (Tab. 2). Based on the entire test period of 105 days, the male turkeys of the probiotic group (EF) consumed 2.7% more feed than the control group (C) birds. This higher consumption was not significant, but nevertheless suggests a stimulatory effect of the probiotic bacteria *Enterococcus faecium*.

Table 2. Influence of the *Enterococcus faecium* preparation on the feed intake of growing male turkeys (g/day/bird)
Tabela 2. Wpływ *Enterococcus faecium* na spożycie paszy u rosnących indorów (g/dzień/szt.)

Period (days) Okres (dni)	Groups/Grupy		SEM	*P*
	C	EF		
0–14	0.023 ±0.001	0.023 ±0.001	0.000	0.359
15–35	0.085 ±0.005	0.088 ±0.003	0.001	0.107
0–35	0.060 ±0.003	0.062 ±0.002	0.001	0.101
36–63	0.246 ±0.009	0.250 ±0.012	0.003	0.443
0–63	0.142 ±0.006	0.148 ±0.007	0.002	0.074
64–77	0.339 ±0.020	0.352 ±0.034	0.007	0.324
0–77	0.181 ±0.011	0.188 ±0.014	0.003	0.258
78–105	0.480 ±0.019	0.489 ±0.022	0.005	0.332
0–105	0.262 ±0.012	0.269 ±0.016	0.003	0.280

Values with different letters differ significantly; ab – $P \leq 0.05$
Średnie oznaczone różnymi literami różnią się istotnie; ab – $P \leq 0,05$

Table 3. Influence of the *Enterococcus faecium* preparation on the body weight of growing male turkeys (kg/bird)
Tabela 3. Wpływ *Enterococcus faecium* na masę ciała rosnących indorów (kg/szt.)

Age of birds (days) Wiek ptaków (dni)	Groups/Grupy		SEM	*P*
	C	EF		
1st	0.058 ±0.001	0.058 ±0.001	0.000	0.862
14th	0.309 ±0.017	0.311 ±0.010	0.003	0.782
35th	1.351 ±0.079	1.398 ±0.047	0.016	0.149
63rd	4.527 ±0.131	4.660 ±0.137	0.035	0.051
77th	6.465^{b} ±0.232	6.747^{a} ±0.301	0.070	0.041
105th	11.001^{b} ±0.226	11.369^{a} ±0.325	0.078	0.013

Values with different letters differ significantly; ab – $P \leq 0.05$
Średnie oznaczone różnymi literami różnią się istotnie; ab – $P \leq 0,05$

Table 4. Influence of the *Enterococcus faecium* preparation on the feed conversion ratio of growing male turkeys (kg feed kg^{-1} BWG)
Tabela 4. Wpływ *Enterococcus faecium* na zużycie paszy rosnących indorów (kg paszy kg^{-1} przyrostu masy ciała)

Period (days) Okres (dni)	Groups Grupy		SEM	*P*
	C	EF		
0–14	1.288 ±0.030	1.304 ±0.020	0.006	0.198
15–35	1.724 ±0.058	1.710 ±0.025	0.010	0.506
0–35	1.638 ±0.045	1.632 ±0.017	0.008	0.746
36–63	2.171 ±0.069	2.165 ±0.091	0.019	0.888
0–63	2.014 ±0.038	2.008 ±0.064	0.012	0.814
64–77	2.456 ±0.101	2.384 ±0.060	0.021	0.083
0–77	2.200 ±0.092	2.167±0.083	0.020	0.438
78–105	2.974 ±0.076	2.983 ±0.135	0.025	0.860
0–105	2.519 ±0.078	2.495 ±0.084	0.019	0.533

Values with different letters differ significantly; ab – $P \leq 0.05$
Średnie oznaczone różnymi literami różnią się istotnie; ab – $P \leq 0,05$

The addition of *Enterococcus faecium* preparation to the feed mixture (group EF) significantly increased ($p \leq 0.05$) the body weight of turkeys in 77^{th} and 105^{th} day of age, respectively by about 4.4 and 3.3% more than the control group – C (Tab. 3). The same tendency was also observed in body weight gain. A lower FCR was observed in group EF (+ *Enterococcus faecium* preparation) in comparison to the control group (C), but the differences in this parameter were not statistically significant (Tab. 4). Throughout the experiment, the animals of group EF to group C consumed 1% less feed per kg gain.

DISCUSSION

Probiotics are alternative substances which have been generally banned in the EU as feed antibiotics since 2006 (EC Regulation No. 1831/2003). As early as 1998, Freitag *et al.* documented the performance-enhancing effect of probiotic preparations in an extensive literature study, especially on piglets, fattening pigs and calves. Poultry was not included in this study. In broiler production by dietary supplementation with probiotic feed additives, higher gains or final body weight, a lower feed conversion ratio and fewer animal losses were also determined [Ghadban 2002]. An analysis of some experiments with turkeys is shown in Table 5. In the majority of the experiments, the turkeys consumed insignificantly less feed in the range of 1 to 2.5%. The increase in body weight gain varied between 0.5 and 4% and was significant only in one experiment. Generally, by using a probiotic preparation in the experimental diets, the feed efficiency of 1.5 to 3.5% was reduced. In the case of this parameter, the differences in the respective control group without probiotic supplementation to the feed are significant in two experiments. Our results were in agreement with those literature data presented in Table 5, especially in the parameters of body weight gain and feed conversion ratio. Männer *et al.* [2002]

also reported a reduction in losses in the probiotics group. Both in the other cited publications as well as in the present own experiment this effect was not observed. In a previous work with probiotic feed additives, the same performance in turkeys was achieved as with antibiotic-supplemented diets [Grashorn 1998].

Table 5. Literature data with different probiotics in meat turkeys (entire experimental period)
Tabela 5. Dane literaturowe na temat różnych probiotyków w żywieniu indyków rzeźnych (cały okres doświadczalny)

Source Źródło	Preparation Preparat	Sex and duration Płeć i okres doświadczenia	Effect (%) compared to control group (= 100) Efekt (%) w porównaniu z grupą kontrolną (= 100)		
			FI spożycie paszy	BWG przyrost masy ciała	FCR zużycie paszy
Buteikis *et al.* 2008	*Pediococcus acidilactici* MA 18/5M (Bactocell®)	Big 6 ♂, 12 weeks	-2	+2	-2
Jeroch *et al*. 2004	*Bacillus cereus toyoi* (ToyoCerin®)	Big 6 ♂, 22 weeks	-2	+2	-2
Männer *et al*. 2002, Männer 2003	*E. faecium* NCIMB 10415 (Cylactin)	Big 6 ♀, 16 weeks	+1	+4	-3.5
		Big 6 ♀, 16 weeks	-2.5	+1	-2
	E. faecium DSM 10663/NCIMB10415 (Oralin)	Big 6 ♀, 16 weeks	-1	+2	-3
		Big 6 ♂, 16 weeks	-1.5	+0.5	-2
	B. licheniformis + *B. subtilis* (BioPlus2B)	Big 6 ♂, 16 weeks	-1	+0.5	-1.5

Although the positive effect of probiotic preparations in farm animals was detected several decades ago, the effectiveness principles of these feed additives has not yet been fully elucidated [Taras *et al*. 2009]. Studies have been conducted into the intestinal flora stabilizing effect of probiotic feed supplements [Vahjen *et al.* 2002, Männer 2003].

CONCLUSIONS

The results of the present experiment are in accordance with other studies on this subject. Probiotics are an alternative to the currently banned food antibiotics in the EU. The use of *Enterococcus faecium*, DSM 10663 NCIMB 10415, can be a performance stabilizing factor in the relatively long fattening period of turkeys.

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Streszczenie. Celem badań było określenie wpływu preparatu probiotycznego zawierającego szczep *Enterococcus faecium*, DSM 10663 NCIMB 10415, na wyniki odchowu młodych indorów podczas doświadczenia trwającego 105 dni, w którym zastosowano 5-fazowy program żywienia. Materiał doświadczalny stanowiło 450 piskląt indyczych płci męskiej, mieszańców BUT Big 6, podzielonych losowo na dwie grupy (9 powtórzeń po 25 ptaków). Wszystkie ptaki karmiono mieszankami izoenergetycznymi i izobiałkowymi. Grupa kontrolna (C) otrzymywała mieszankę bazową, a w grupie doświadczalnej (EF) dietę bazową uzupełniano preparatem z *Enterococcus*

faecium w ilości $1{,}05 \times 10^9$ jtk kg^{-1} paszy (30 g t^{-1} paszy). Określono następujące cechy odchowu: masa ciała, spożycie paszy oraz współczynnik wykorzystania paszy. Dodanie preparatu probiotycznego zawierającego *Enterococcus faecium* do mieszanki grupy doświadczalnej (EF) istotnie ($p \leq 0{,}05$) zwiększyło masę ciała indyków określoną w 77. i 105. dniu życia – odpowiednio o około 4,4 i 3,3%, w porównaniu z grupą kontrolną (C). Taką samą tendencję obserwowano w odniesieniu do przyrostów masy ciała ptaków. Wyniki doświadczenia potwierdzają wcześniejsze doniesienia naukowe dotyczące stosowania probiotyków i ich korzystnego wpływu na cechy produkcyjne. Probiotyki stanowią alternatywę dla antybiotyków paszowych, których stosowania zakazano w krajach Unii Europejskiej. Zastosowanie *Enterococcus faecium*, DSM 10663 NCIMB 10415, może wpływać stabilizująco na wyniki produkcyjne indyków podczas stosunkowo długiego okresu odchowu.

Słowa kluczowe: indyki, probiotyk, *Enterococcus faecium*, wyniki odchowu