The effect of *Bacillus cereus* var. *toyoi* and avilamycin on the faecal microflora of turkeys

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ABSTRACT

The effect of *Bacillus cereus* var. *toyoi* spores and avilamycin as bacterial and antibiotic growth promoters, respectively, on faecal microflora of turkeys was determined. The experiment was carried out on 108 turkeys randomly allocated to 3 groups: control and avilamycin- or *Bacillus* spore-treated. After 12 weeks of feed supplementation and a subsequent 4-week period following withdrawal, selected groups of faecal microflora were determined, and the results were compared with those in the control group.

Neither avilamycin nor *B. cereus* var. *toyoi* spores affected *Bifidobacterium* or *Lactobacillus* counts, whereas both supplements significantly increased the counts of *E. coli* by 0.84 and 0.69 log cycle ($P \le 0.05$ for both). *B. cereus* var. *toyoi* caused a significant increase in counts of spores of both aerobic and anaerobic proteolytic bacteria by 0.98 ($P \le 0.001$) and 0.83 ($P \le 0.01$) log cycle, respectively.

At the end of the period following withdrawal, in the avilamycin group, counts of *Bifidobacterium* and *Lactobacillus* significantly decreased by 1.14 ($P \le 0.01$) and 1.97 ($P \le 0.001$) log cycle, respectively, as did the counts of anaerobic proteolytic bacteria spores, which decreased by 1.18 ($P \le 0.001$). In the *B. cereus* var. *toyoi* group, however, increased counts of *Lactobacillus*, by 0.91 ($P \le 0.01$), *E. coli*, by 0.71 ($P \le 0.05$), anaerobic proteolytic bacteria spores, by 1.37 ($P \le 0.001$), and of anaerobic saccharolytic bacteria spores, by 0.72 ($P \le 0.05$) log cycle were found.

The tested growth promoters did not affect the groups of beneficial gut microflora, whereas they stimulated the growth of opportunistic bacteria.

The performance parameters of turkeys, better in groups administered avilamycin and *B. cereus* var. *toyoi*, were comparable with the control at the end of period after withdrawal.

KEY WORDS: turkeys, B. cereus var. toyoi, avilamycin, intestinal microflora

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INTRODUCTION

The number of antibiotics approved for use in animal feeding is being successively reduced, and now, in most European Union countries, only two of them, flavophospholipol and avilamycin, are allowed for poultry as growth promoters (Garshorn, 1998; Hughes and Heritage, 2002). The results of our preliminary studies suggest it may be possible to replace antibiotics with carefully selected probiotics for protection against zoonotic pathogens and enhancement of intestinal physiological functions (Bielecka et al., 2002a,b). One of the probiotics offered for farm animals is the spores of *Bacillus cereus* var. *toyoi* (CNCM I 1012 / NCIB-40112), a strain originally isolated from soil. The preparation of *B. cereus* var. *toyoi* spores is intended for use with sows and growing pigs, laying hens and broiler chickens, rabbit does and growing rabbits, calves and beef cattle at application rates of ~1 g/kg (i.e. ~1 × 10⁹ cfu/kg) of complete feedstuff. According to the declaration of the distributor, the addition of *B. cereus* var. *toyoi* has no adverse effect on the gastrointestinal microflora of any target species.

The best documented trends resulting from the addition of *B. cereus* var. *toyoi* to the diets were improved growth performance of piglets fed *B. cereus* var. *toyoi* to two months of age and a significant improvement in the survival rate and the weaning weight of piglets when the reproductive sows received that preparation (Report of SCAN, 2001). Other effects observed in some trials with animals fed spores of *B. cereus* var. *toyoi* included reduced severity of diarrhoea, decreased morbidity in piglets challenged with *E. coli* O149K and K99, improvement in average daily gain and feed conversion ratio (FCR). Some results also indicate that *B. cereus* var. *toyoi* has an effect on the morphology of the pig intestine, increasing the villus length in the small intestine and decreasing the number of goblet cells with 2,6-sialylated mucins in the large intestine (Baum et al., 2002).

The reason for undertaking the current study was the contradictory opinions about the usefulness of *B. cereus* var. *toyoi* as a probiotic in animal feeding expressed in publications, overinterpretation of some scientific data in commercial leaflets, as well as insufficient scientific confirmation of the usefulness of *B. cereus* var. *toyoi* in turkey nutrition. The aim of the study was to determine the effect of *B. cereus* var. *toyoi* and avilamycin as, respectively, bacterial and antibiotic growth promoters on selected performance indices as well as on the faecal microflora of turkeys.

MATERIAL AND METHODS

The experiments were carried out on 108 males of BUT-9 turkeys randomly assigned to 3 groups (3 pens in each). The birds were housed under the environ-

mental and lighting conditions recommended by Faruga and Jankowski (1996) and fed *ad libitum* with mash diets (Table 1). The turkeys in the control group were receiving the basal diet, devoid of growth promoters. Avilamycin was administered from the 1st to the 8th week in the amount of 8 mg/kg and from the 9th to the 12th week in the amount of 6 mg/kg of feed, whereas *B. cereus* var. *toyoi* spores were used for twelve weeks at a dose of 150 mg/kg. Both diets were lacking these additives from week 13 to 16 of the experiment. The considered indices of turkey performance were live body weight (LBW) determined on weeks 4, 8, 12, and 16, FCR, and mortality.

TABLE 1

	Feeding period, weeks					
Component, %	0 - 4	5 - 8	9 - 12	13 - 16		
Wheat	46.05	53.40	61.13	66.32		
Soyabean meal (46% CP)	41.00	34.00	26.00	19.50		
Meat meal (55% CP)	8.00	8.00	8.00	8.00		
Soyabean oil	0.90	1.00	0.80	0.45		
Lard	-	-	0.70	2.65		
Remaining ¹	4.05	3.60	3.37	3.08		
Calculated						
crude protein	288.2	263.40	234.90	209.80		
ME, MJ/kg	11.68	11.97	12.39	12.95		
Lys	18.40	16.10	13.60	11.50		
Met + Cys	11.40	10.60	9.90	9.30		
Thr	11.60	10.30	9.30	7.50		
Ca	13.50	12.50	11.90	11.20		
P available	7.50	7.00	6.60	6.40		

Composition and calculated nutritive value of basal diet, g/kg

¹ limestone, monocalcium phosphate, NaCl, NaHCO₃, L-lysine HCl, DL-methionine, L-treonine, mineral - vitamin premix in amount adequate to requirement, feed enzyme, from 1 to 8 weeks only Diclazuril (1 mg/kg)

On weeks 12 and 16, i.e. at the end of administration of avilamycin and *B. cereus* var. *toyoi*, and at the end of the experiment, excrement was collected from 12 and 10 turkeys, respectively, from each group and its microflora analysed. All bacterial determinations were done immediately after sampling. Excrement samples of 1-2 g were weighted and mixed with 1% peptone water containing 0.5% (w/v) meat peptone (Peptobak, BTL, Łódź, Poland) and 0.5% (w/v) pancreatic casein hydrolysate (bio-Trypcase, bioMérieux, pH 7.0), as a diluter. After dispersion, serial decimal dilutions were made, avoiding aeration.

The live cell count of bifidobacteria was determined on modified Garche's nutrient agar medium (Rasic, 1990) (1 L distilled water contained: 20 g meat peptone, 2 g yeast extract, 0.4 g L-cysteine hydrochloride, 10 g lactose, 6 g CH₃COONa, 0.12 g MgSO₄ × 7H₂O, 2.5 g Na₂HPO₄ × 12H₂O, 2 g KH₂PO₄, pH 6.5±0.1) after

incubation at 37° C/72 h under anaerobic conditions in Anaerobic System (Oxoid) with Gas Pak CO₂+H₂ (Linegal Chemicals GmbH, Poland). Their identification was based on the appearance of colonies, the specific morphology of cells was checked under a Microphot FXA (Nikon, Japan) phase contrast microscope; the presence of an enzyme specific for bifidobacteria, fructose-6-phosphate phosphoketolase (F6PPK - EC 4.1.2.22) (Scardovi, 1986), was determined in different morphological cell types.

Lactobacilli were counted on MRS agar medium (pH 6.4 ± 0.1) using a double-layer technique, incubation at 37°C/72 h and microscopic confirmation of cell morphology.

The most probable number (MPN) of *E. coli* was determined using brilliant green bile broth, after incubation at 37°C/48 h, subsequent transfer of positive samples into tryptone water, followed by overnight incubation at 44°C and indole test (Collins et al., 1995).

Spores were determined after heating of adequate dilutions at 80°C/20 min and cooling. Anaerobic saccharolytic bacteria spores were counted as MPN using tubes containing nutrient agar with 1% glucose, 1% yeast extract and 1% peptone (fortified SPC) (Harrigan and Mc Cance, 1976) with pyrogallol stoppers (incubation at 37°C up to 7 days); the number of anaerobic proteolytic bacteria spores was determined in tubes using broth gelatine (incubation at 22-25°C up to 5 days) and gelatine stoppers; spores of aerobic proteolytic bacteria, on plates with fortified SPC agar supplemented with milk (incubation at 37°C 24-48 h).

The results of the performance studies were subjected to statistical analysis using analysis of variance and STAT-1 software. The microbiological results were expressed as log colony forming units cfu/g excrement or MPN/g. Arithmetic means in groups of turkeys as well as significance of differences between groups were calculated with Student's *t*-test for less numerous groups.

RESULTS

The LBW of 4- and 8-week-old turkeys was significantly higher (P<0.01) in the group administered avilamycin than in the others (Table 2). Twelve-week-old turkeys from that group were still significantly heavier (P<0.05) in comparison with the control. At the end of the experiment, the LBW of the 16-week-old turkeys was not significantly differentiated, showing only a tendency to be higher in the groups administered avilamycin and *B. cereus* var. *toyoi* (with means 11.08 kg and 10.94 kg, respectively) than in the control (10.66 kg). The FCR value was the lowest in the group of turkeys fed with the antibiotic growth promoter (2.54 kg/kg LBW), and was lower by 0.13 and 0.10 kg than in the control and in the group receiving *B. cereus* var. *toyoi*, respectively. Two and one

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	Group			
Indices	C	A	В	SEM
Live body weight, kg				
at weeks				
4	0.91 ^A	1.01 ^B	0.94 ^A	0.013
8	3.07 ^A	3.58 ^B	3.25 ^A	0.049
12	7.24ª	7.84 ^b	7.55 ^{ab}	0.098
16	10.66	11.08	10.94	0.156
FCR, kg/kg LBW	2.67	2.54	2.64	0.008
Mortality, pieces	-	2	1	

Performance of turkeys

C - control, A - fed diets with avilamycin, B - fed diets with B. cereus var. toyoi

a,b,A,B means in rows with no common superscripts were significantly differed at: a,b P≤0.05; A,B P≤0.01

mortalities per 36 individuals in avilamycin- and *Bacillus* groups, respectively, occurred.

After 12 weeks of the experiment, the *Bifidobacterium* and *Lactobacillus* counts were determined as markers of gut microflora beneficial to host health, the *E. coli* count as opportunistic or potentially pathogenic bacteria, and the spore count as an indicator of aerobic and anaerobic spore-forming bacteria of different biochemical properties, proteolytic or saccharolytic. The next determination was performed 4 weeks after withdrawal of avilamycin and *B. cereus* var. *toyoi* from the diet, i.e. on week 16 of the experiment.

At the end of supplementation of the diet, in the control group, the populations of *Lactobacillus* and *Bifidobacterium* had the highest counts with means of $8.76 \pm 0.32 \log \text{cfu/g}$ and $8.72 \pm 0.43 \log \text{cfu/g}$, respectively (Figure 1). The *E. coli* count (mean $5.67 \pm 0.79 \log \text{MPN/g}$) was lower by ~3 log cycles than of *Lactobacillus* and *Bifidobacterium*. The lowest were the counts of spores: $2.21 \pm 0.37 \log$ cfu/g for aerobic proteolytic bacteria spores, and $2.23 \pm 0.91 \log \text{cfu/g}$ for anaerobic spores. Among the spores, anaerobic saccharolytic bacteria spore counts (mean $1.09 \pm 0.40 \log \text{MPN/g}$) were the lowest.

In the groups of turkeys fed the diets with avilamycin or *B. cereus* var. *toyoi*, *Bifidobacterium* counts equalled 8.59 ± 0.90 and $8.76 \pm 0.53 \log \text{cfu/g}$, *Lactobacillus* counts, 9.18 ± 0.61 and $8.74 \pm 0.44 \log \text{cfu/g}$, respectively. Neither were significantly different in comparison with the control. In contrast, in both avilamycin- and *B. cereus* var. *toyoi*-fed groups, the *E. coli* counts, 6.50 ± 0.87 and $6.36 \pm 0.53 \log \text{MPN/g}$, were significantly higher, by 0.84 and 0.69 log cycle, than those in the control (both P ≤ 0.05), respectively. Proteolytic bacteria spore counts in the avilamycin group were $2.22 \pm 0.58 \log \text{cfu/g}$ for aerobes and $2.81 \pm 0.46 \log \text{cfu/g}$ for anaerobes. They remained on a level comparable with their controls. In contrast, their counts in the *B. cereus* var. *toyoi* group, at a level of 3.19 ± 0.38





 \circ - counts in single samples

 \blacksquare - means <u>+</u>SD

groups of turkeys: C - control, A - administered with avilamycin, B - administered with *B. cereus* var. *toyoi*

counts different from the control at significance level: $*P \le 0.05$, $**P \le 0.01$, and $***P \le 0.001$



Figure 2. Faecal microflora of turkeys at the 16th week of the experiment, i.e. 4 weeks after deprivation of avilamycin and *B. cereus* var. *toyoi* from diet

 \circ - counts in single samples

 \blacksquare - means <u>+</u>SD

groups of turkeys: C - control, A - administered with avilamycin, B - administered with *B. cereus* var. *toyoi*

counts different from the control at the significance level: $*P \le 0.05$, $**P \le 0.01$, and $***P \le 0.001$

TABLE 3

Crown					Spores of	
Gloup	Bifido	Lacto	E coli	proteolytic	proteolytic	saccharolytic
01 turlcaria	-bacterium	-bacillus	E. COII	aerobic	anaerobic	anaerobic
turkeys			bacteria	bacteria	bacteria	
С	0.27	↓ 0.80***	0.33	0.24	↓ 0.75*	0.17
А	↓ 1.14**	↓ 1.97***	0.38	0.18	↓ 1.18***	0.18
В	0.22	0.13	0.34	↓ 0.54*	0.20	↑ 1.07***

Changes in microflora from the 12th to 16th week of the experiment, i.e. within 4 weeks after deprivation of avilamycin and *B. cereus* var. *toyoi* from diets

increase \uparrow or decrease \downarrow of bacterial count, $\Delta \log cfu$ (or MPN)/g group of turkeys significance level: *P ≤ 0.05 ; **P ≤ 0.01 ; ***P ≤ 0.001

C - control, A - fed diets with avilamycin, B - fed diets with B. cereus var. toyoi

for aerobes and $3.05 \pm 0.33 \log$ cfu/g for anaerobes, were significantly higher, by 0.98 (P \leq 0.001) and 0.83 log cycle (P \leq 0.01), than in the control. The counts of anaerobic saccharolytic bacteria in groups administered avilamycin or *B. cereus* var. *toyoi* were 1.54 ± 0.83 and $0.91 \pm 0.52 \log$ MPN/g, respectively, and were not significantly different from the control.

On week 16 of the experiment, the bacteria counts in the control were as follows: *Bifidobacterium* $8.45 \pm 0.86 \log$ cfu/g, *Lactobacillus* $7.96 \pm 0.41 \log$ cfu/g, *E. coli* $5.99 \pm 0.53 \log$ MPN/g, aerobic proteolytic bacteria spores $2.45\pm0.49 \log$ cfu/g, anaerobic proteolytic bacteria spores $1.47\pm0.23 \log$ cfu/g, and anaerobic saccharolytic bacteria spores $1.26\pm0.48 \log$ MPN/g (Figure 2). In comparison with the data from the 12th week, only two groups of bacteria, *Lactobacillus* and proteolytic anaerobic bacteria spores were significantly decreased, by 0.80 (P ≤ 0.001) and 0.75 log cycle (P ≤ 0.05), respectively (Table 3).

On week 16, in the group from which avilamycin was withdrawn, the determined counts were as follows: *Bifidobacterium* 7.45±0.48 log cfu/g, *Lactobacillus* 7.21±0.50 log cfu/g, *E. coli* 6.13±0.81 log MPN/g, aerobic proteolytic bacteria spores 2.41±0.57 log cfu/g, anaerobic proteolytic bacteria spores, 1.63±0.39 log cfu/g, and anaerobic saccharolytic bacteria spores 1.61±0.50 log MPN/g (Figure 2). The majority of bacterial populations were not affected by withdrawal of avilamycin, except for a significant decrease of *Bifidobacterium* populations by 1.00 log cycle (P≤0.05) in comparison with the control, and a tendency for the *Lactobacillus* count to decrease (by 0.75 log cycle; P≤0.1). In comparison with the data from week 12, the populations of three groups of bacteria were significantly reduced: *Bifidobacterium* by 1.14 (P≤0.01), *Lactobacillus* by 1.97 (P≤0.001), and anaerobic proteolytic bacteria spores by 1.18 (P≤0.001) log cycle (Table 3).

On week 16, in the group deprived of *B. cereus* var. *toyoi*, the determined counts were as follows: *Bifidobacterium* $8.54\pm0.53 \log \text{cfu/g}$, *Lactobacillus* $8.87\pm0.43 \log \text{cfu/g}$, *E. coli*, $6.70\pm0.58 \log \text{MPN/g}$, aerobic proteolytic bacteria spores 2.64 ± 0.47

log cfu/g, anaerobic proteolytic bacteria spores 2.85 ± 0.31 log cfu/g, and anaerobic saccharolytic bacteria spores 1.98 ± 0.55 log MPN/g (Figure 2). In comparison with the control, increased levels of *Lactobacillus* by 0.91 log cycles (P \leq 0.01), *E. coli* by 0.71 log cycle (P \leq 0.05), anaerobic proteolytic bacteria spores by 1.37 log cycle (P \leq 0.001), and anaerobic saccharolytic bacteria spores by 0.72 (P \leq 0.05) were observed. In relation to the data from week 12, the withdrawal of *B. cereus* var. *toyoi* resulted in a significant decrease in the count of aerobic proteolytic bacteria spores, by 0.54 log cycle (P \leq 0.05) and a significant increase in the count of anaerobic saccharolytic bacteria spores, by 1.07 log cycle (P \leq 0.001) (Table 3).

DISCUSSION

A meaningful positive influence of the antibiotic growth promoter on the body weight gain was observed in the first 8 weeks of feeding. In the subsequent periods this effect was gradually reduced. The effect of *B. cereus* var. *toyoi* spores did not reach statistical significance, although a positive tendency towards enhancement was observed, and was rather stable during administration. The greatest influence of the antibiotic growth promoter on BWG of turkeys was also found by Plavnik and Wax (1998) during the first eight weeks of life, whereas Garshorn (1998) did not observe any significant differences between turkeys fed diets without a growth promoter as compared with an antibiotic (flavophospholipol) or *B. cereus* var. *toyoi*.

Neither avilamycin nor *B. cereus* var. *toyoi* affected the counts of *Bifidobacterium* and *Lactobacillus*, intestinal bacteria beneficial to host health, nor the counts of spore-forming saccharolytic bacteria during supplementation. On the contrary, they both significantly increased the counts of *E. coli*. Avilamycin had no influence on the count of proteolytic spore-forming bacteria, either aerobic or anaerobic, whereas *B. cereus* var. *toyoi* significantly increased their counts. All these changes ranged from 0.7 to 1.0 log cycle.

A significant increase in the *E. coli* count by 0.69 log cycle in the *B. cereus* var. *toyoi* group contradicted the leaflet information that *E. coli* cells are systematically eliminated from the intestines. In several studies on the efficacy of *B. cereus* var. *toyoi*, no changes of microflora were observed, although on occasion a decrease in the numbers of *E. coli* was reported, accompanied by a small increase in lactobacilli counts (Report of SCAN, 2001). Kyriakis et al. (1999) used spores of *B. cereus* var. *toyoi* for effective control of post-weaning diarrhoea syndrome of piglets caused by an enterotoxigenic strain of *Escherichia coli*.

The significantly increased count of aerobic proteolytic bacteria spores might include the introduced spores of *Bacillus cereus* var. *toyoi*. Perhaps the germinated spores through their vital activity modified the intestinal environment in a

direction favourable for anaerobic proteolytic bacteria, the number of which was also increased in the group of turkeys fed the diet with *B. cereus* var. *toyoi*. If the spores of *Bacillus cereus* var. *toyoi* are able to germinate and temporarily colonise the anaerobic intestinal environment, another explanation is possible entailing the anaerobic metabolism, as suggested by Casula and Cutting (2002) for the probiotic strain of *Bacillus cereus* in murine intestines.

Our results partially resemble those of Gedek et al. (1993) who showed no effect of *B. cereus* FH1457 administered to piglets in the amount of 10^7 , 10^8 , or 10^9 cfu/kg of feed during two periods of a 42-day feeding trial, 21 days each, on the counts of *Lactobacillus/Bifidobacterium*, *Eubacteria*, and *Bacteroidaceae* in the duodenum, jejunum, ileum, caecum, and colon. In different periods of the trial and in different parts of the gastrointestinal tract, the counts of *E. coli* and *Enterococcus* increased or decreased. The *B. cereus* count increased only at the highest dosage used in that experiment.

After withdrawal of avilamycin from the diet, some unfavourable changes occurred. A significant decrease in *Bifidobacterium* and *Lactobacillus* populations was observed, especially with reference to their counts at the end of the supplementation period, but also in comparison with the control at week 16. Such changes did not happen after withdrawal of *B. cereus* var. *toyoi*–the *Bifidobacterium* and *Lactobacillus* counts were unchanged from weeks 12 to 16 of the experiment, moreover, on week 16, the *Lactobacillus* count was higher than in the control group.

During the experiment the spore counts generally did not exceed $10^{4}/g$ with means from 10^1 to $10^3/g$. It is obviously difficult to imagine that a dormant life form, especially in low amounts, may exhibit any probiotic effect, which points to vegetative cells as the active agents, although scientific opinions are divergent. On the basis of studies on non-pathogenic strains of B. subtilis, B. cereus and B. clausii used as probiotics, Spinosa et al. (2000) suggested that the spores of *Bacillus* are the forms responsible for any claimed probiotic effect, as they did not find detectable amounts of vegetative cells in intestinal samples. The opposite point of view was expressed by Jadamus et al. (2001). The authors observed repeated germination and sporulation of B. cereus var. tovoi during intestinal passage and concluded that the spores germinated rapidly in broiler chickens and piglets, which was a necessary prerequisite for their possible probiotic effects. Moreover, germination and *in vivo* sporulation of vegetative cells indicated that the probiotic strain was metabolically active in the intestine of both animal species. That opinion was also confirmed by Hoa et al. (2001) and Casula and Cutting (2002) for probiotic strains of B. subtilis in mice.

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CONCLUSIONS

The levels of microflora in the control and avilamycin supplemented group suggest that administration of antibiotic growth promoter did not improve the profile of faecal microflora, moreover, it decreased the counts of beneficial bacteria, of *Bifidobacterium* (significantly) and *Lactobacillus* (a tendency) in the period after their withdrawal. Performance was better during feeding supplemented diets, but it was not different at the end of the study. The spores of *B. cereus* var. *toyoi* did not alter the level of beneficial intestinal bacteria in comparison with the control, except for a significantly higher count of *Lactobacillus* after being withdrawn, whereas they increased the counts of *E. coli* and proteolytic spores, both during administration and after withdrawal. Generally, the microbiological results of *B. cereus* var. *toyoi* supplementation seemed to be better than avilamycin, especially at the end of the period following their withdrawal, whereas performance parameters, better in groups during administration of avilamycin and *B. cereus* var. *toyoi*, were comparable with the control after these supplements were withdrawn.

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STRESZCZENIE

Wpływ Bacillus cereus var. toyoi i avilamycyny na mikroflorę odchodów indyków

Badano wpływ przetrwalników Bacillus cereus var. toyoi i avilamycyny, stosowanych jako stymulatory wzrostu, na mikroflorę odchodów indyków. Doświadczenie przeprowadzono na 108 indykach losowo podzielonych na 3 grupy; kontrolną oraz otrzymujące avilamycynę lub przetrwalniki Bacillus. Po 12 tygodniach podawania tych preparatów i następnym 4-tygodniowym okresie bez ich stosowania oznaczano wybrane grupy drobnoustrojów, a wyniki odnoszono do uzyskanych w grupie kontrolnej. Stwierdzono brak istotnego wpływu avilamycyny i B. cereus var. tovoi na liczebność populacji Bifidobacterium i Lactobacillus, natomiast istotne zwiększenie liczby E. coli, odpowiednio o 0,84 ($P \le 0,05$) i 0,69 ($P \le 0,05$) log NPL/g. Ponadto B. cereus var. toyoi powodował zwiększenie liczby przetrwalników proteolitycznych bakterii tlenowych i beztlenowych odpowiednio o 0,98 (P \leq 0,001) i 0,83 (P \leq 0,01) log jtk/g. Po zaprzestaniu podawania avilamycyny istotnie obniżyła się liczebność *Bifidobacterium* i *Lactobacillus*, odpowiednio o 1,14 ($P \le 0.01$) i 1,97 $(P \le 0.001)$ log jtk/g oraz przetrwalników beztlenowych bakterii proteolitycznych o 1,18 log jtk/g $(P \le 0.001)$. Po zaprzestaniu podawania *Bacillus cereus* var. *tovoi* stwierdzono istotne zwiekszenie liczby Lactobacillus o 0,91 log jtk/g ($P \le 0,01$), E. coli o 0,71 log NPL/g ($P \le 0,05$), przetrwalników beztlenowych bakterii proteolitycznych o 1,37 log jtk/g ($P \le 0,001$) i przetrwalników beztlenowych bakterii sacharolitycznych o 0,72 log NPL/g ($P \le 0,05$). Badane stymulatory wzrostu nie wpływały na korzystną mikroflorę jelitową, natomiast stymulowały wzrost bakterii oportunistycznych. Wyniki odchowu indyków, lepsze w grupach otrzymujących avilamycyne i B. cereus var. tovoi, były porównywalne z uzyskanymi w grupie kontrolnej po zaprzestaniu suplementacji.