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The Influence of Probiotic Bacteria (Bacillus Toyoi) on Livability and Performance of Young Meat-Type Turkeys

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ABSTRACT

The aim of study was to determine the impact of a probiotic preparation containing live bacteria *Bacillus cereus var. toyoi* on the performance of turkeys in order to define the effective dose of the preparation and to evaluate its influence on poults' livability in the first month of life. Three hundred and sixty B.U.T. 9 male turkey were divided into three groups. The control group (I) was fed a diet with no probiotic additive, and groups II and III were given a probiotic product containing $0.2x10^9$ and $1x10^9$ *B. toyoi* CFU/kg of feed respectively. Body weight was recorded in weeks 1, 6, and 18. Livability, feed conversion ratio, and European Efficiency Factor (EEF) were estimated. In experiment II, the impact of a standardized probiotic dose on livability and performance parameters of B.U.T. 9 turkey poults were evaluated. Two groups were selected within each sex: control (C), feed without probiotic, and experimental (T), which feed contained the probiotic preparation. Mortality and body weight were registered. The better livability and performance of the turkeys fed probiotic justifies the use of bacteria *Bacillus cereus var. toyoi* to improve performance results and livability during the first weeks of the turkeys' life. The bacterium strain concentration of $1.0x10^9$ CFU/kg of feed can be assumed as optimal.

INTRODUCTION

The mortality of turkey poults under modern farming conditions practice causes economic losses for farmers. Some of the factors that may influence poult mortality are breeder flock age, genetic strain, as well as the hatching process, characteristics of the farm on which birds were reared, and even temperature during transportation of the poults to the farm (Carver *et al.,* 2000; 2002). The critical period, when the highest mortality occurs, corresponds to the first 4-6 weeks of life in young meat-type turkeys, during which poults begin to present wattle growth. During this period, poults are highly sensitive to adverse environmental conditions and to nutrient deficiencies. Considering also that many farmers rear turkey poults with the purpose of sale only to the end of critical period, the first weeks of rearing determine production profitability. Therefore, methods to improve poult live ability are needed.

In the past, due to the progressive intensification of animal husbandry, in-feed antibiotics were the main group of preparations used to stabilize the specific composition of the intestinal microbiota and to protect animals from infections, therefore functioning as growth promoters. However, due to potential risk of bacterial resistance spread and other

negative consequences for consumers, the use of in-feed antibiotics in the feed of monogastric animals was banned (Regulation EC No 1831/2003). Currently, there is an increasing body of research for alternative additives with similar effects, such as probiotics (Anadón *et al.,* 2006; Bomba *et al*., 2002). Their mode of action consists of the competitive exclusion of pathogenic microflora; production of organic acids, mainly lactic acid (lowering pH of the intestinal content); neutralization of enterotoxins produced by pathogenic strains of *E. coli*; and production of antibiotic-like substances against pathogenic microbiota, such as nizine, reuterin, acidophilin and acidolin (Jin *et al.*, 1997; Selvet *et al.,* 2012).

The ultimate goal of the commercial application of probiotics is to increase economic profitability as a result of a demonstrable improvement of animal performance, reduction of morbidity and mortality in the animals and of human pathogenic bacterial populations (Flint & Garner, 2009). Spore-forming bacteria, especially of the genus *Bacillus*, have been shown to prevent gastrointestinal diseases in animals and humans. They have many applications, such as treating immunosuppressive and antibiotic-associated diarrhea. A scientific study also showed the tolerance of probiotic *Bacillus* spp. strains to different physiological conditions, as well as the inhibition of *Salmonella Typhimurium* (Menconi *et al.*, 2013).

Bacillus cereus var. *toyoi* (*B. toyoi*) is a strain isolated from the soil. It does not produce diarrheal or emetic enterotoxins and it is not a genetically modified organism. Toyocerin® is a preparation of viable spores of *B. cereus* var. *toyoi* is used as a probiotic microorganisms in the feed to promote growth and digestive health (Wiliams *et al.*, 2009).

Two experiments were conducted. The first one experiment (I) was carried out under experimental conditions, and aimed at evaluating the effects of a probiotic additive containing live bacteria *Bacillus cereus var. toyoi* on the performance of meat-type turkeys and determining its most effective dose. The second study (II) was performed under standard farming and production conditions, to evaluate the impact of the dietary addition on turkey poult health during the first four weeks of their life.

MATERIAL AND METHODS

The farm, where both experiments were conducted, remained under constant supervision and care of a veterinarian. The causes of deaths and culling during the rearing period were routinely analyzed for possible infectious diseases. The feedstuffs used for the formulation of both the control and the probiotic-containing feeds derived from the same batches. Feeds were analyzed for basic nutrients contents, and metabolizable energy was calculated according to the equation provided in the European Tables (1989). The mash feed containing viable *Bacillus cereus toyoi* was analyzed for the active ingredient in the German laboratory Bayrisches Landesamt für Gesundheit und Lebensmittelsicherheit (Oberschleiaheim, Germany).

Experiment I

In total, 360 one-d-old B.U.T. 9 medium weight turkey males were individually identified and divided into three experimental groups, with four replicates each. The birds were kept in a closed house on deep litter, using typical intensive farming microclimatic conditions. Birds were fed *ad libitum*. Group I, considered as the control group (C), was fed a standard balanced basal diet with no in-feed antibiotic growth promoters, but with the inclusion of a coccidiostatic drug. Groups II and III were fed the basal diet containing different levels of probiotic additive inclusion, of $0.2x10^9$ and $1x10^9$ *Bacillus toyoi* CFU/kg of feed in groups II and III, respectively (Table 1).The probiotic was added to the basal feed in the mixer, together with the main ingredients.

The basal diet was formulated for five different rearing phases (Table 2), according to the recommendations of the NRC (1994).

Metabolizable energy and crude protein levels of the experimental diets, according to feeding phase, are shown in Table 2. Birds were weighed at the beginning of the experiment (1-day-old poults), and with 6 and 18 weeks of age. Feed intake and livability were recorded. Feed conversion ratio and European Efficiency Factor (EEF) were calculated. The formula used to calculate the EEF was: (final bird weight, kg × livability, %)/(age, days × feed conversion ratio × 100).

Table 1 – Experimental feeds in experiment I

Group I	Basal feed with no antibiotic growth promoter (control group)
Group II	Basal feed + $0.2x10^9$ *B. toyoi* CFU/kg of feed
Group III	Basal feed + $1x10^9$ *B. toyoi* CFU/kg of feed

(CFU) – Colony Forming Units

Experiment II

A flock of 12,000 B.U.T. 9 turkey poults of both sexes at 1:1 ratio reared up to slaughter was used. Birds were distributed into 24 pens of 500 birds each.

Table 2 – Nutritional value of mixture (in 1kg) used in turkey feeding

	Prestarter (wk 0-3)	Starter (wk 4-6)	Grower 1 (wk 7-9)	Grower 2 (wk 10-12)	Grower 3 (wk 13-18)
Metabolizable energy (kcal/kg)	2839	2905	2980	3054	3142
Crude protein (%)	26.7	25.0	22.8	21.2	18.9
Coccidiostatic (Monensin, mg)	100	100	100	80	-

Birds ere submitted to same management and feeding practices as those in experiment I. Based on the results of Experiment I, the dietary inclusion level of $1x10^9$ *Bacillus cereus var. toyoi* CFU/kg of feed was determined as the effective dose of the probiotic product. Birds of each sex were divided in two groups. The control group (C) was fed a basal diet with no the addition of in-feed antibiotics or other growth promoters, and containing a coccidiostatic drug. Experimental group (T) was fed the basal feed with the inclusion of the probiotic product. Birds were evaluated up to 4 weeks. The number of dead and culled birds, as well as body weight, were recorded during this period.

The data from both experiments were analyzed using statistical package SPSS 12.0PL (SPSS, 2003) by single factor analysis of variance. Means were compared by Duncan's test. The non-parametric χ^2 test was used to analyze mortality data of both experiments.

RESULTS

Experiment I

The main part of this experiment was published (Grela *et al.,* 2009). In this paper, only the unpublished results are considered, and they constituted the basis for the second research. Due to large amount of data, the results were divided into two subperiods: the so-called critical period, up to six weeks of age, and from seven weeks of age to the end of the rearing (18 weeks). Table 3 presents the performance results of turkey males in trial I.

Body weight was significantly different among treatments during both subperiods of the study. The birds from group III, fed with $1x10^9$ *B. toyoi* CFU/kg feed, were the heaviest. The feed conversion ratio (FCR) of birds in groups II and III was significantly better compared with the control group experiments during both subperiods. Average FCR was 3.4% lower in group III and 2.3% in lower in group II relative to the control group during the entire rearing period. The highest European Efficiency Factor value was obtained in group III, which was fed with $1x10^9$ FCU *B. toyoi*/kg of feed, reaching 427 points after 18 weeks of rearing. Group II, which was fed with a smaller probiotic, and the control group presented about 4.2% and 11.2% lower FCR relative to group III. It should be noted that a higher European Efficiency Factor value was calculated for group fed the highest probiotic level relative the control group already in week 6.

Table 4 shows livability results. Livability rates were high and not statistically different among all groups. However, the highest value was obtained in group III (97.7%), followed by group II, which livability rate was 96.2% until week 6. It should be noted that after week six turkeys' livability reached 100%.

Table 3 – Production effects of turkey males in experiment I

Parameter	Groups			SEM	p-value
	I	II	III		
Initial body weight (g)	56.9	56.7	56.8	0.080	0.351
Body weight (g)					
Up to week 6	$2,226^a$	$2,242^a$	$2,301^a$	7.97	0.000
weeks 7-18	$11,236^a$	$11,425^b$	$11,602^c$	27.5	0.000
Total	$13,462^a$	$13,666^b$	$13,905^c$	27.8	0.020
Feed conversion ratio					
Up to week 6	1.68	1.66	1.61	0.014	0.108
Weeks 7-18	2.80^b	2.74^a	2.72^a	0.012	0.001
Average	2.62^c	2.56^b	2.53^a	0.011	0.000
European Efficiency Factor					
Up to week 6	299^a	318^{ab}	341^b	6.641	0.017
Weeks 7-18	478^a	497^b	509^c	4.195	0.000
Total	379^a	409^b	427^b	6.016	0.020

Group I: basal feed with no antibiotics (control group), group II: basal feed + $0.2x10^9$ B. toyoi CFU/kg of feed, group III: basal feed + $1x10^9$ B. toyoi CFU/kg of feed

Table 4 – Livability rate of turkey males in experiment I (%)

Time (wk)	Groups			χ^2 (p-value)
	I	II	III	
0-6	92.4	96.2	97.7	0.953
7-18	100	100	100	1.000
Total	92.4	96.2	97.7	0.953

Group I: basal feed with no antibiotics (control group), group II: basal feed + 0.2x10^9 *B. toyoi* CFU/kg of feed, group III: basal feed + 1x10^9 *B. toyoi* CFU/kg of feed.

Experiment II

This experiment involved a number of birds more than 30 times higherthan in experiment I. In the control groups, the number of dead and culled birds were not statistically significant between sexes (Table 5). However, when the birds were fed the probiotic additive, this number was lower in females than in males. Statistically significant difference in average number of dead birds per pen in each group was demonstrated. Non-parametric as well as parametric test confirmed positive effect of *Bacillus cereus var. toyoi* addition on livability of both sexes of birds.

Table 6 shows the body weight of male and female turkeys measured in II experiment. After four weeks of rearing, turkeys fed the probiotic product tended to be slightly heavier, regardless of sex; however, these differences were not statistically significant.

DISCUSSION

The effectiveness of probiotics depends on the type of bacterial strains and concentration of microorganisms added to the feed during their formulation. Toyocerin®, a preparation containing the bacterial strain *Bacillus toyoi*, has shown beneficial effects when administered to pigs (Jadamus *et al.*, 2002; Stamati *et al.,* 2006; Papatsiros *et al.*, 2011; Kantas *et al.*, 2014), rabbits (Brzozowski & Strzemecki, 2013), Japanese quails (Homma & Shinohara, 2004), chickens and turkeys (Jeroch *et al.*, 2004; Grela *et al.,* 2009; Novak *et al.*, 2011).

The higher body weight of the turkeys fed the probiotic product detected in experiment I was confirmed by many authors. In the study of Blair *et al.* (2004), turkeys fed a mixture containing aprobiotic microorganism (*Bacillus subtilus*) or an antibiotic growth promoter presented higher weight gain during 18 weeks of rearing compared with those fed a basal diet with no additives. Torres-Rodriguez *et al*. (2007) obtained significantly higher body weight and lower production costs associated with any necessary treatment in female turkeys fed a probiotic (*Lactobacillus spp.*) additive, particularly when flocks from farms presenting historical poor performance and health. In our study, the body weight difference amounted to about 3% between the control and experimental group fed with the higher probiotic dose and was statistically significant, despite presenting similar health status. In another study (Kozłowski *et al.*, 2014), the addition of a *Enterococcus faecium* preparation to the feed significantly increased the body weight of turkeys in 3.3 to 4.4%.

Salmonella infection is considered the main cause of economic losses in poultry production. It has been shown that the administration of a probiotic product, also in combination with an organic acid, considerably reduced the number of *Salmonella* spp. excreted by turkeys (Vincente *et al.*, 2007). It was proven that a mixture containing 11 probiotic strains of lactic-acid bacteria maybe effective for the prevention of infections caused by *Salmonella enterica* in chicks and turkey poults (Menconi *et al*., 2011). *Salmonella enterica*is the bacterial zoonotic factor most frequently transferred from poultry product to humans. However, the use of probiotic *Bacillus* bacteria reduces *Salmonella* colonization, as well as increases body weight gain in chicks and poults (Shivaramaiah *et al.*, 2011). The lower mortality observed in the groups fed

Table 5 – Livability of male and female turkeys in experiment II (%).

Sex	♂♂		♀♀		SEM	p-value
Groups	C	T	C	T		
Number of dead and culled birds (average per pen)	25.3^c	17.5^b	24.8^c	13.5^a	1.081	0.000
Livability (%)	94.93^a	96.50^b	95.03^a	97.30^c	0.216	0.000
						χ^2 (p-value)
Total number of dead and culled birds	152	105	149	81	-	0.000

Group I: basal feed with no antibiotics (control group), group II: basal feed + 0.2x10^9 *B. toyoi* CFU/kg of feed, group III: basal feed + 1x10^9 *B. toyoi* CFU/kg of feed.

Table 6 – Body weight of male and female turkeys measured at the beginning and in week 4 in experiment II (g).

Time (wk)	Groups ♂♂ C	♂♂ T	♀♀ C	♀♀ T	SEM	*p-value*
0	55.7	56.0	55.0	55.0	*0.013*	*0.351*
4	1148	1204	1024	1079	*0.624*	*0.144*

with probiotic additive in the present study indicates that *Bacillus cereus* var. *toyoi* in hibited *Salmonella* spp. replication and positively influenced bird's health. In the experiments of Vilá *et al.* (2009), the dietary addition of Toyocerin also reduced the prevalence of *Salmonella* in poultry and, in the case of broiler chickens, it also significantly improved performance at slaughter age.

Livability of turkeys is lowest during first weeks of their rearing. One of the reasons for this early mortality may be insufficient morphological and functional development of the gastrointestinal tract (GIT). However, GIT development can be stimulated by appropriate nutritional supplements (Lecewicz *et al.,* 2008). Studies indicate that administration of probiotics can accelerate the maturation of turkey poults'GIT, suggesting the possibility of using probiotics as growth promoters, including in organic production systems due to their natural origin (Moyle *et al.,* 2012). The results obtained by Timmerman *et al.* (2006) suggest that probiotics may be useful to reduce early turkey early mortality. Mortality in broilers was reduced by the addition of chicken-specific probiotics (consisting of seven *Lactobacillus* species) to the drinking water, and was associated with a 4.87% increase in final body weight. Amer & Khan (2012) observed significantly lower mortality in a group of chickens fed a diet with probiotic supplementation (*Lactobacillus acidophilus, Bacillus subtilis, Saccharomyces cerevisiae*, and *Aspergillus oryzae*) in comparison with those fed an antibiotic or a control diet. Using two types of lactic acid probiotic bacteria (*Pediococcus* spp.) in feeds, Brzoska *et al.* (2010) detected significantly lower mortality in the supplemented broilers relative to the controls. These observations were confirmed by our results; however, the differences observed in the present study were larger (sometimes the double) of the rates obtained in the cited papers. Toyocerin® also positively influenced the mortality rate in other animal species, particularly when combined with benzoic acid (Papatsiros *et al*., 2011).

In conclusion, the results of the present study demonstrate that the preparation containing *Bacillus cereus var. toyoi* probiotic strains can be used to improve turkey performance, but its effectiveness depends on the dose of the preparation. The concentration $1.0x10^9$ CFU/kg feed can be assumed as the optimal dose. Additionally, it was shown that the additive containing *Bacillus cereus var. toyoi* administered to turkey poults reared under standard intensive rearing conditions significantly improves the livability of birds during the first weeks of their life and maybe recommended as a health and growth promoter for turkeys.

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