The effect of a new probiotic preparation on the performance and faecal microflora of broiler chickens

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ABSTRACT: The aim of the study was to examine the possibility of replacing antibiotic growth promoters with a new probiotic preparation. One gram of the preparation contains: 4.7×10^7 of LAB (*Lactobacillus casei/paracasei* LOCK 0920, *L. brevis* LOCK 0944, *L. plantarum* LOCK 0945), 2.0×10^3 of yeast (*Saccharomyces cerevisiae* LOCK 0140) and a 50 mg of extract from Yucca Schidigera. The experiment was conducted on 99 female broilers divided into three groups consisting of 33 chickens, kept separately. A wheat- and soybean meal-based diet was divided into three parts: A (containing 1 g/kg of probiotic for starter and grower diets and 0.5g/kg for finisher diet), B (containing 14 mg/kg of flavomycin), and C (unsupplemented). The diets were fed from Day 1 to Day 41 of life. Final BW was on average 2.4 kg in all groups, FCR was 1.63 kg feed/kg BWG, neither BWG nor FCR nor mass of the liver, pancreas and gastrointestinal tract were significantly influenced by supplementing the diet with either additive. Special attention should be drawn to the fact that supplementing feeds with a probiotic preparation already after one week of breeding considerably decreased the number of *Clostridium* bacteria in broilers' faeces. Nevertheless, it should be emphasized that in this study the excreta of chickens fed with the feed mixed with a probiotic contained the lowest changeability of the number bacteria of the *Enterobacteriaceae* family and bacteria belonging to *coli* groups in individual weeks of breeding. It may be concluded that the studied probiotic can be considered as a substitute for antibiotic growth promoters in broiler diets.

Keywords: probiotic; broiler chickens; faecal microflora; performance

The 20th century is marked by an intensified vegetable and animal production which is necessary to meet the demand for food of the continually growing human population, especially for animal proteins. Intensified food production requires the use of various additives to feed which promote animal growth processes. Food additives include substances which are not indispensable for life and correct development of animals; however, added to the diet they support digestion and absorption of nutrients and have a beneficial effect on the general state of animal health. The most important feed additives comprise, among others, antibiotics, probiotics, enzymes and amino acids.

On 1st January 2006 the European Union introduced a complete ban on the use of antibiotic growth promoters in feeds for animals for consumption. The ban was introduced at the same time in all Member States. Since then, antibiotics have been allowed to be used as medicines only in medical feeds or prophylactic additives. Resolution No 1831/2003 EC of the European Parliament and Council of 22nd August 2003 devoted to the issue of additives used in feeding animals described probiotics as alternative feed additives to antibiotic growth promoters (Casewell et al., 2003; Patterson and Burkholder, 2003; Berghmann et al., 2005). According to the legislative framework of the FAO and WHO (Anonymous, 2002) probiotics are "live microorganisms that, administered in adequate amounts, confer health benefits to the host". The use of probiotic organisms in order to

sustain appropriate homeostasis of the digestive tract and protect it against pathogenic microflora is a common practice in poultry production in some parts of the world (Verstegen and Williams, 2002), especially in Japan and Europe (McEwen, 2001; McEwen and Fedorka-Cray, 2002; Philips et al., 2003; Patterson and Burkholder, 2003).

MATERIAL AND METHODS

Therefore, the aim of this research was to examine the possibility of replacing antibiotic growth promoters with a probiotic preparation. One gram of the preparation contains: 4.7×10^7 of LAB (*Lactobacillus casei/paracasei* LOCK 0920, *L. brevis* LOCK 0944, *L. plantarum* LOCK 0945), 2.0×10^3 of yeast (*Saccharomyces cerevisiae* LOCK 0140) and 50 mg of extract from Yucca Schidigera. The strains mentioned above come from The Pure Cultures Collection of Industrial Microorganisms (LOCK 105) of the Technical University in Lodz. They are resistant to gastric juice and bile activity and they manifest high fermenting ability.

The experiment was conducted on 99 female broilers divided into three groups consisting of 33 chickens, kept separately (Institute of Animal Physiology and Nutrition, Polish Academy of Sciences in Jablonna). A wheat- and soybean meal-based diet (Table 1) was divided into three parts: A (containing 1 g/kg of probiotic for starter and grower diets and 0.5 g/kg for finisher diet), B (containing 14 mg/kg of Flavomycin), and C (unsupplemented). The diets were fed from 1 to 41 day of life. The uneaten leftovers of the feed, as well as the chickens themselves, were weighed at weekly intervals. After the experiment all the birds were slaughtered. Twenty birds were selected from each group and their livers, pancreas, as well as the digesta from their crops, stomachs and gizzards, jejunums, ileums and caeca were all weighed. The pH values of individual sections of the digestive tract were measured (pH-meter WTW pH/340, slides pH WTW D-82362). The content of ammonia in plasma was measured (apparatus Vitros, slides NH_3DT) as well. On the basis of the results achieved the following parameters were defined: feed consumption, Body Weight Growth, Feed Consumption Ratio and European Broiler Index (EIB).

Fresh excreta samples were taken from five chickens per group at weekly intervals. Excreta were suspended in buffered 1% peptone water (1 : 9 w/v), after which serial decimal dilutions were prepared. The following bacterial species were identified: total number of bacteria on Plate Count agar and aerobic incubation at 30°C/48 h; *Lactobacilli* on MRS agar medium and anaerobic incubation at 37°C/48 h; *Enterobacteriaceae* on VRBD agar and aerobic incubation at 37°C/24 h; *coli* group on McConkey agar and aerobic incubation at 37°C/24 h; *Enterococcus* on Esculine Bile agar and aerobic incubation at 35°C/72 h and *Clostridium* on TCS agar and anaerobic incubation at 37°C/24 h. Each determination was done in triplicate. The results are presented as colony forming units (CFU) per gram of excreta.

The results were subjected to one-way analysis of variance using Anova; Origin ver. 6.1 software.

Table 1. Composition of a starter, grower and finisher feed for broiler chickens (g/kg)

Components	Starter	Grower	Finisher
Wheat	330.70	379.00	400.60
Soy pellets	380.60	332.80	304.00
Corn	200.00	200.00	200.00
Fodder chalk	8.50	8.50	8.50
Dicalcium phosphorus	18.00	18.00	16.00
NaCl	3.00	3.00	3.00
Rapeseed oil	50.00	50.00	60.00
Vitamin-mineral premix	5.00	5.00	5.00
Wheat starch or probiotic	1.00	1.00	0.50
L-lysine (78%)	1.00	0.80	0.20
DL-methionine (98%)	1.20	1.40	1.20
Feed enzyme	1.00	1.00	1.00

RESULTS AND DISCUSSION

On the basis of the acquired results it was calculated that the final body weight of chickens on their 41st day of life was on average 2.4 kg in all groups, with the use of 1.63 kg of feed per 1 kg of the body mass growth. The deaths (3.4%) that occurred during the 1st week of life were not related to the experimental factor (Table 2). European Broiler Index was very high in all groups and was approximately 360. No statistically significant differences were noted between the different kinds of feed supple-

Table 2. Results of breeding broiler chickens

Feeding period Diets group	Feed consumption		BWG		FCR kg feed/kg BWG (kg/kg ± SD)	Body weight averaged (g ± SD)	EIB
	(g ± SD)	(g/day)	(g ± SD)	(g/day)			
1–21 day of life (Starter)							
A	1 065 ± 62	50.5	815 ± 46	38.8	1.31 ± 0.05	852 ± 46	
B	1 093 ± 84	52.0	833 ± 65	44.4	1.31 ± 0.04	870 ± 65	
C	1 049 ± 60	49.9	816 ± 48	38.8	1.29 ± 0.05	853 ± 48	
21–35 day of life (Grower)							
A	1 879 ± 141	134.2	1 071 ± 89	76.5	1.75 ± 0.12	1 923 ± 106	
B	1 901 ± 189	135.8	1 079 ± 113	77.1	1.76 ± 0.10	1 949 ± 154	
C	1 887 ± 183	134.8	1 096 ± 121	78.3	1.73 ± 0.11	1 948 ± 140	
35–41 day of life (Finisher)							
A	878 ± 54	146.3	465 ± 86	77.6	1.95 ± 0.38	2 388 ± 131	
B	873 ± 47	145.6	455 ± 42	75.8	1.93 ± 0.18	2 404 ± 150	
C	879 ± 31	146.5	450 ± 60	75.0	1.99 ± 0.30	2 398 ± 128	
Entire feeding period							
A	3 822 ± 201	93.2	2 351 ± 131	57.3	1.63 ± 0.08	2 388 ± 131	357.4
B	3 867 ± 295	94.3	2 367 ± 150	57.7	1.63 ± 0.06	2 404 ± 150	359.7
C	3 815 ± 217	93.1	2 361 ± 128	57.6	1.62 ± 0.07	2 398 ± 128	361.1

A = supplemented with probiotic, B = supplemented with antibiotic, C = unsupplemented, SD = standard deviation
EIB – European Broiler Index = 100 × the final body weight (kg) × survivability (%)/age (days) × FCR (kg/kg)

mentation and the breeding parameters, i.e., the Body Weight Growth and the Feed Consumption Ratio. However, in the group of broilers fed with the feed mixed with probiotic supplement the birds' body weight was the most stable during individual breeding periods, which is proved by a lower standard deviation (SD). Irrespective of the kind of supplement added (a probiotic, an antibiotic or none), the relative body weights of the birds' livers, pancreas and abdominal fat pad, as well as of individual sections of the gastrointestinal tract converted into percentage of the chickens' body weight before slaughter were similar and statistically insignificant (Table 3). Similar results were acquired

Table 3. Relative weight of liver, pancreas, abdominal fat pad and individual sections of the digestive tract converted into percentages of the body weight before slaughter

Group	LBW (g)	Liver (% LBW)	Pancreas (% LBW)	Abdominal fat pad (% LBW)	Gastrointestinal tract weight (% LBW)				
					crop	stomach	jejunum	ileum	caeca
A	2 500	2.51	0.17	1.06	0.70	1.85	1.43	0.73	0.33
B	2 557	2.37	0.17	1.12	0.66	1.76	1.42	0.70	0.36
C	2 557	2.51	0.16	0.96	0.66	1.83	1.39	0.71	0.33

A = supplemented with probiotic, B = supplemented with antibiotic, C = unsupplemented

Table 4. NH_3 in blood and pH of digesta in 41-day old broilers

Group	NH_3 (μmol/l)	Digesta pH in				
		crop	stomach	jejunum	ileum	caeca
A	187	4.66*	4.13	5.71	6.67*	6.53
B	161	4.51*	4.22	5.62	6.20*	6.56
C	173	4.88*	4.10	5.85	6.80*	6.68

*means are significantly in each addition ($P < 0.05$)

in the research conducted by Watkins and Kratzer (1983, 1984) and Maiolino et al. (1992). Jin et al. (1998) proved that once the broilers' diet was supplemented with *L. acidophilus* or a mix of *Lactobacillus* bacteria, i.e., *L. acidophilus* (2), *L. fermentum* (3). *L. crispatus* (1) and *L. brevis* (6), it did not have any statistically significant influence on the weight of crops, livers, liens, duodenums and small intestines converted into percentage of the chickens' body weight before slaughter either. Similar results were also reported by Fethiere and Miles (1987) as well as by Watkins and Kratzer (1984).

The concentration of ammonia in the broilers' blood varied, depending on the kind of the feed additive used (Table 4). The highest concentration of 187 μm/l was found in the blood of chickens fed with the feed containing a probiotic. The lowest one, 161 μm/l, was reported in the blood of birds fed with feed mixed with an antibiotic. Nevertheless, it should be emphasized that the concentration of ammonia in the blood of all groups of broilers was within the physiological norms. Irrespective of the kind of the supplements added to feeds, the changes in pH of the chyme in the stomach, jejunum and caecum were not statistically significant (Table 4). It was seen, however, that supplementation of the feed with a Flavomicin antibiotic led to a decrease in the pH of the digesta found in the birds' crops and ileums. The pH of the digesta in these parts of the digestive tract equaled 4.88 and 6.80, respectively. After supplementation of the feed with a probiotic preparation the pH in these sections was insignificantly lower and equaled 4.66 and 6.67, respectively. Jin et al. (1998) found a statistically significant decrease in the pH ($P < 0.05$), in comparison with the control group, in groups of chickens receiving feed with the addition of *L. acidophilus* or a mix of *Lactobacillus* bacteria. However, this referred only to caecum.

On the basis of the microbiological research conducted no statistically significant differences were found between broilers fed with the feed supplemented with a probiotic, an antibiotic or with no supplementation and between the general count of bacteria in the faeces of chickens in individual weeks of breeding, i.e., from 10^9 to 10^{11} CFU/g (Figure 1). The average number of *Lactobacillus* bacteria in the faeces of all the groups of birds examined in individual weeks of breeding ranged from 10^9 to 10^{10} CFU/g (Figure 2). The analysis of

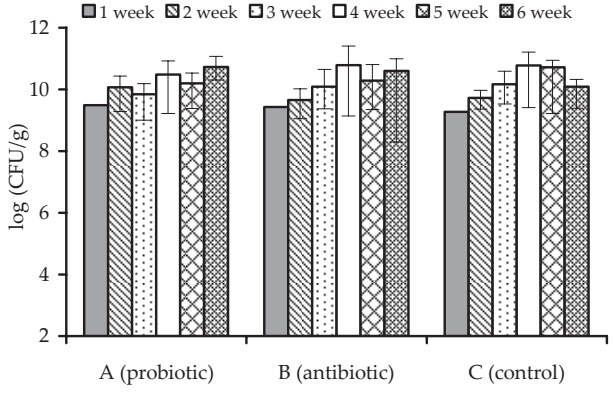

Figure 1. Total number of bacteria in the faeces of chickens

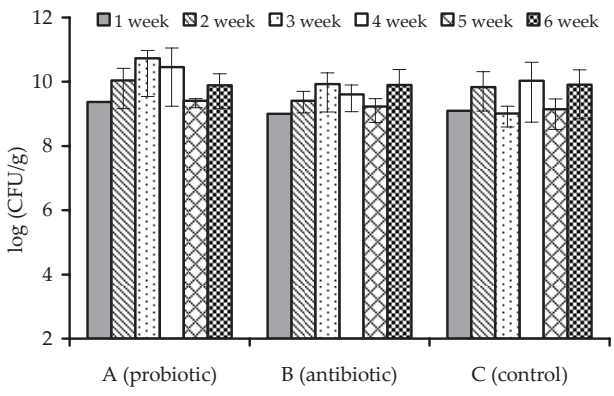

Figure 2. *Lactobacillus* bacteria in the faeces of chickens

the data collected showed statistically significant differences ($P < 0.01$) only after the third week of breeding between the kind of feed additive used and the number of bacteria of *Lactobacillus* genus in faeces. Jin et al. (1998) demonstrated that when the broilers received a supplement of *L. acidophilus* or a mix of bacteria of the *Lactobacillus* genus, it did not effect any statistically significant increase in the number of *Lactobacillus* strains in caecum during individual weeks of breeding. As for the small intestine, significant changes were only noted on the 30th day of breeding. Watkins and Kratzer (1983, 1984) did not find any significant increase in the number of bacteria of *Lactobacillus* sp. in the chickens' intestines either. Similarly, there were no statistically significant differences found between the kind of feed supplementation and the number of *Enterobacteriaceae* bacteria in the excreta samples during individual weeks of breeding, which ranged from 10^7 to 10^8 CFU/g (Figure 3). The number of c*oli* group bacteria in the faeces of broilers receiving the feed supplemented with a probiotic or the one with no supplementation ranged from 10^6 to 10^7 CFU/g throughout various weeks of the experiment. As for the third group (fed with the feed containing an antibiotic), the value ranged from 10^6 to 10^8 CFU/g. The analysis of the data gathered showed that only after the third week of breeding did there appear statistically significant differences between the kind of feed supplementation and the number of *coli* bacteria in the excreta samples (Figure 4). Kralik et al. (2004) reported a decrease in the number of bacteria of the *Enterobacteriaceae* family and *coli* strains, to about 90% of the control sample; i.e., 1.39×10^6 and 2.72×10^5 CFU/g, after 42 days of supplementing water with a probiotic containing 5×10^9 CFU/g of *Enterococcus faecium* M-74. However, they did not find any statistically significant differences in relation to bacteria of *Staphylococcus* sp., *Bacillus* sp. and *Clostridium* sp. Jin et al. (1998) claimed that adding *L. acidophilus* or a mix of *Lactobacillus* bacteria into chickens' diet induced a statistically significant ($P < 0.05$) decrease in the number of c*oli* group bacteria in caecum in relation to the control sample, however, only on days 10 and 20 of breeding. They did not report similar findings for the small intestine. Corresponding research findings in this respect were presented by Francis et al. (1978). Nevertheless, it should be emphasized that in this study the excreta of chickens fed with the feed mixed with a probiotic contained the lowest changeability of the number of bacteria of the *Enterobacteriaceae* family and bacteria belonging to the *coli* group in individual weeks of breeding. The highest changeability was discovered in the faeces of broilers receiving unsupplemented feed. Special attention should be drawn to the fact that supplementing feeds with a probiotic preparation considerably decreased the number of *Clostridium* bacteria in broilers' faeces already after one week of breeding. The number of these bacteria was ca. 10^5CFU/g, and in the remaining two groups included in the research it was two orders of magnitude higher (Figure 5). After the second week of breeding, regardless of the kind of feed supplementation, a decrease (equaling one order of magnitude) in the number of bacteria belonging to the *Clostridium* genus in the excreta of chickens from individual groups was noted. After the third week of breeding a further reduction in the number of the above-mentioned bacteria was observed only in

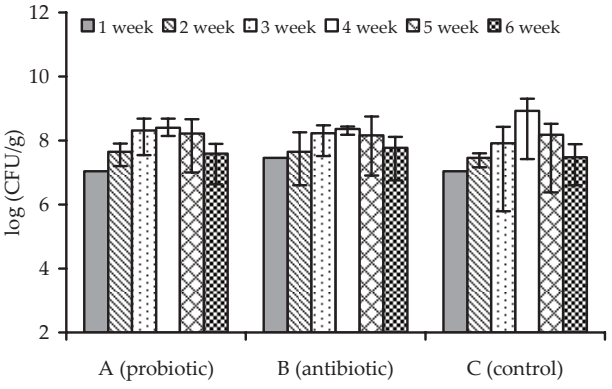

Figure 3. *Enterobacteriaceae* bacteria in the faeces of chickens

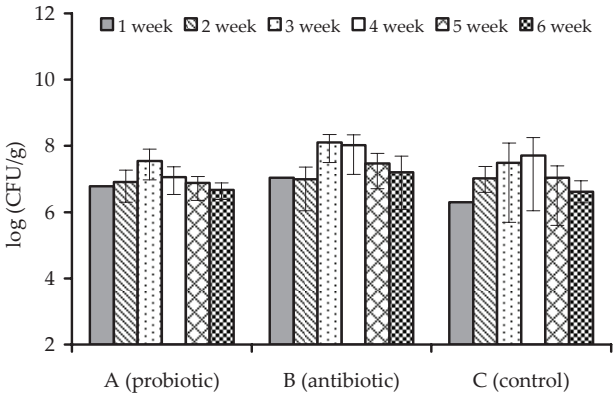

Figure 4. Total number of *coli* groups in the faeces of chickens

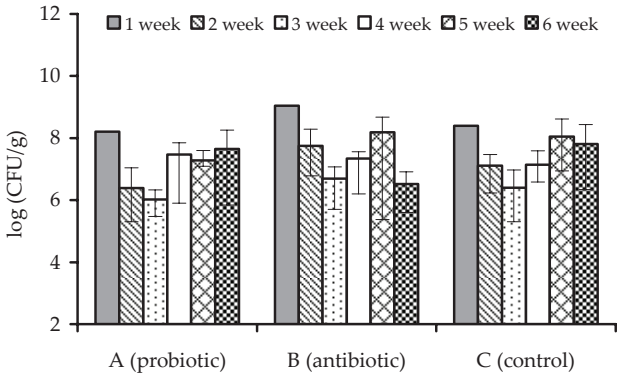

Figure 5. *Enterococcus* bacteria in the faeces of chickens

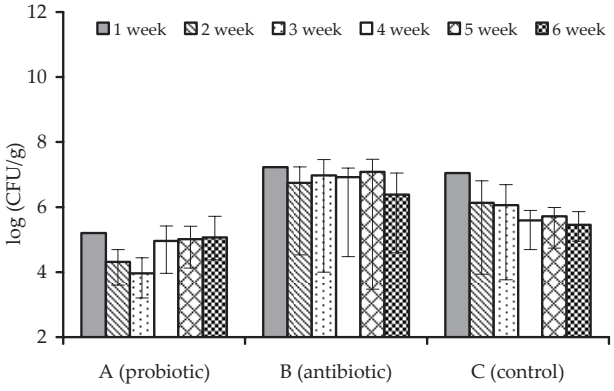

Figure 6. *Clostridium* bacteria in the faeces of chickens

the faeces of broilers fed with the feed containing a probiotic additive. The number was approximately 10^3 CFU/g and it was three orders of magnitude lower in comparison to the result obtained in the case of chickens from the remaining two groups. After the fourth week of breeding, depending on the kind of supplementation, we observed diversification of the number of that kind of bacteria ranging from 10^4 CFU/g to 10^5 CFU/g. It should be stated, though, that still the lowest number (10^4 CFU/g) of microorganisms in question was found in the excreta of the birds fed with the feed containing a probiotic. After the fifth and sixth week of breeding the number of *Clostridium* bacteria in the faeces of broilers fed with the probiotic-supplemented feed and with the unsupplemented one was at a stable level and stood at 10^5 CFU/g. The group receiving the feed supplemented with an antibiotic, in comparison with the other two groups, was still characterised by the highest number of these bacteria. It was two and one order of magnitude higher, respectively. This outcome is important as, since antibiotic growth promoters were banned from use in feeds for poultry, the number of intestinal problems in the case of such birds bred for consumption is likely to increase, especially those connected with *Clostridium perfringens* bacteria (necrotic enteritis – NE). In France, for instance, the incidence of NE increased from 4.0% in 1995 to 12.4% in 1999. Similar trends were observed in other European countries.

CONCLUSIONS

On the basis of this study it may be concluded that a probiotic preparation containing in one kg: 4.0×10^{10} of *Lactobacillus* bacteria, 4.0×10^6 of yeasts *Saccharomyces cerevisiae* and 50 g of the extract from Yucca Schidigera, may successfully replace antibiotic growth promoters previously used in poultry breeding.

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