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EFFECT OF DIETARY PROBIOTIC CONTAINING *LACTOBACILLUS* BACTERIA, YEAST AND YUCCA EXTRACT ON THE PERFORMANCE AND FAECAL MICROFLORA OF BROILER CHICKENS

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Key words: probiotic, antibiotic growth promoter, faecal microflora, broiler chickens

The experiment was carried out on 99 female broilers, allocated to 3 groups of 33 birds, kept in individual cages. A wheat- and soyabean meal-based diet was formulated and divided into 3 batches: P (supplemented with a probiotic composed of lactic acid bacteria, yeasts and yucca extract), A (supplemented with flavomycin) and C (unsupplemented). Diets were fed from day 1 to 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 the hereby research excreta of chickens fed with the feed mixed with a probiotic contained the lowest changeability of the number bacteria of *Enterobacteriaceae* family and bacteria belonging to *coli* group in individual weeks of breeding. It may be concluded that the studied probiotic can be considered to be a substitute for antibiotic growth promoters in broiler diets.

INTRODUCTION

Antibiotics that for many years have been used as growth promoters have greatly improved the economic efficiency of slaughter animals production. However, they have also, and perhaps even in the first place, been conducive to the emergence of more and more numerous bacterial strains with antibiotic-resistance features. That is why the number of antibiotics admitted to use in feeds as growth promoters has gradually been decreased [Mc Ewen, 2001; Mc Ewen & Fedorka–Cray, 2002; Philips *et al*., 2003]. On 1 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. The Resolution No 1831/2003 EC of the European Parliament and Council of 22 August 2003 devoted to the issue of additives used in feeding animals enumerated probiotics as feed additives alternative to antibiotic growth promoters [Casewell *et al*., 2003; Patterson & Burholder, 2003; Berghmann *et al*., 2005]. According to the definition accepted by FAO and WHO [2002] probiotics are defined as "live microorganisms that served in proper amounts to ensure health benefits to the host". Using probiotic organisms in order to sustain appropriate homeostasis of digestive tract and protect it against pathogenic microflora is a common practice in poultry production in some parts of the world [Verstegen & Williams, 2002], especially in Japan and Europe [Patterson & Burkholder, 2003].

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

MATERIALS AND METHODS

The experiment was conducted on 99 female broilers divided into 3 groups consisting of 33 chickens, kept separately (Institute of Animal Physiology and Nutrition, Polish Academy of Sciences in Jabłonna). A wheat- and soybean meal-based diet (Table 1) was divided into 3 parts; P (containing 1 g/kg of probiotic for starter and grower diets and 0.5 g/kg for finisher diet), A (containing 14 mg/kg of Flavomicin) 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 in weekly intervals. After the experiment all the birds were slaughtered. The researchers selected 20 birds from each group and took out and weighed their livers, pancreas as well as the digesta from their crops, stomachs

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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

and gizzards, jejunums, ileums and caeca. 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 Gain, Feed Consumption Ratio and European Broiler Index (EIB).

Fresh excreta samples were taken from 5 chickens per group at weekly intervals. Excreta were suspended in buffered 1% peptone water (1:9 w/v), then serial decimal dilutions were prepared. The following bacteria 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.

RESULTS AND DISCUSSION

On the basis of the acquired results it was stated that the final body weight of chickens on their 41 day of life equaled 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 it equaled *ca*. 360. No statistically significant differences were noted as for the kind of feed supplementation and the breeding parameters; *i.e.* the Body Weight Gain 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 lower

TABLE 2. Results of breeding broiler chickens.

Feeding period Diets Group	Feed consumption		BWG		FCR kg feed/kg BWG	Body weight averaged	EIB
	g±SD	g/day	g±SD	g/day	kg/kg±SD	g±SD	
1 – 21 day of life (Starter)							
P	1065±62	50.5	815±46	38.8	1.31±0.05	852±46	
A	1093±84	52.0	833±65	44.4	1.31±0.04	870±65	
C	1049±60	49.9	816±48	38.8	1.29±0.05	853±48	
21 – 35 day of life (Grower)							
P	1879±141	134.2	1071±89	76.5	1.75±0.12	1923±106	
A	1901±189	135.8	1079±113	77.1	1.76±0.10	1949±154	
C	1887±183	134.8	1096±121	78.3	1.73±0.11	1948±140	
35 – 41 day of life (Finisher)							
P	878±54	146.3	465±86	77.6	1.95±0.38	2388±131	
A	873±47	145.6	455±42	75.8	1.93±0.18	2404±150	
C	879±31	146.5	450±60	75.0	1.99±0.30	2398±128	
Entire feeding period							
P	3822±201	93.2	2351±131	57.3	1.63±0.08	2388±131	357.4
A	3867±295	94.3	2367±150	57.7	1.63±0.06	2404±150	359.7
C	3815±217	93.1	2361±128	57.6	1.62±0.07	2398±128	361.1

P – supplemented with probiotic; A – supplemented with antibiotic; C – unsupplemented; SD – standard deviation; EIB – European Broiler Index = 100×the final body weight (kg)×survivability (%)/age (days)×FCR (kg/kg) standard deviation (SD). It was stated that irrespectively 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% of the chickens' body weight before slaughter were similar and statistically insignificant (Table 3). Similar results were acquired in the research conducted by Watkins & 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 bacteria of *Lactobacillus* kind, *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% of the chickens' body weight before slaughter either. Similar outcome was also presented by Fathiere & Miles [1987] as well as by Watkins & Kratzer [1984].

The concentration of ammonia in the broilers' blood was varied, depending on the kind of the feed additive used (Table 4). The highest concentration equaling 187 μm/L was found in the blood of chickens fed with the feed containing a probiotic. The lowest one, equaling 161 μm/L was reported in the blood of birds fed with the feed mixed with an antibiotic. Nonetheless, it should be emphasized that the concentration of ammonia in the blood of all groups of broilers fitted within the physiological norms. Irrespectively 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 stated, though, 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 additive 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 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 faeces of all the groups of birds examined in individual weeks of breeding equaled from 10^9 to 10^{10} CFU/g (Figure 2). The analysis of 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] proved that when the broilers received a supplement of *L. acidophilus* or a mix of bacteria of *Lactobacillus* genus, it did not influence 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 & 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 equaled from 10^7 to 10^8 CFU/g (Figure 3). The number of *Coli* group bacteria in the faeces of broilers receiving the feed supplemented with a probiotic or the one with no supplementation equaled 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) it equaled from 10^6 to 10^8 CFU/g. The analysis of the data gathered showed that only after the third week of breeding there occurred some statistically significant differences between the kind of feed supplementation and the number of bacteria of *coli* group in the excreta samples (Figure 4). Kralik *et at.* [2004] reported a decrease in the number of bacteria of the *Enterobacteriaceae* family and the *coli* strains, equaling *ca*. 90% in relation to 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, he did not find any statistically significant differences in relation to bacteria of *Staphylococcus* sp., *Bacillus* sp. and *Clostridium* sp. Jin *et al.*

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
P	187	4.66^{ab}	4.13	5.71	6.67^{a}	6.53
A	161	4.51^{b}	4.22	5.62	6.20^{b}	6.56
C	173	4.88^{a}	4.10	5.85	6.80^{a}	6.68

^{ab} means in columns with no common superscripts were significantly different at $p<0.05$

TABLE 3. Relative weight of liver, pancreas, abdominal fat pad and individual sections of the digestive tract calculated into % 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
P	2500	2.51	0.17	1.06	0.70	1.85	1.43	0.73	0.33
A	2557	2.37	0.17	1.12	0.66	1.76	1.42	0.70	0.36
C	2557	2.51	0.16	0.96	0.66	1.83	1.39	0.71	0.33

P – supplemented with probiotic; A – supplemented with antibiotic; C – unsupplemented

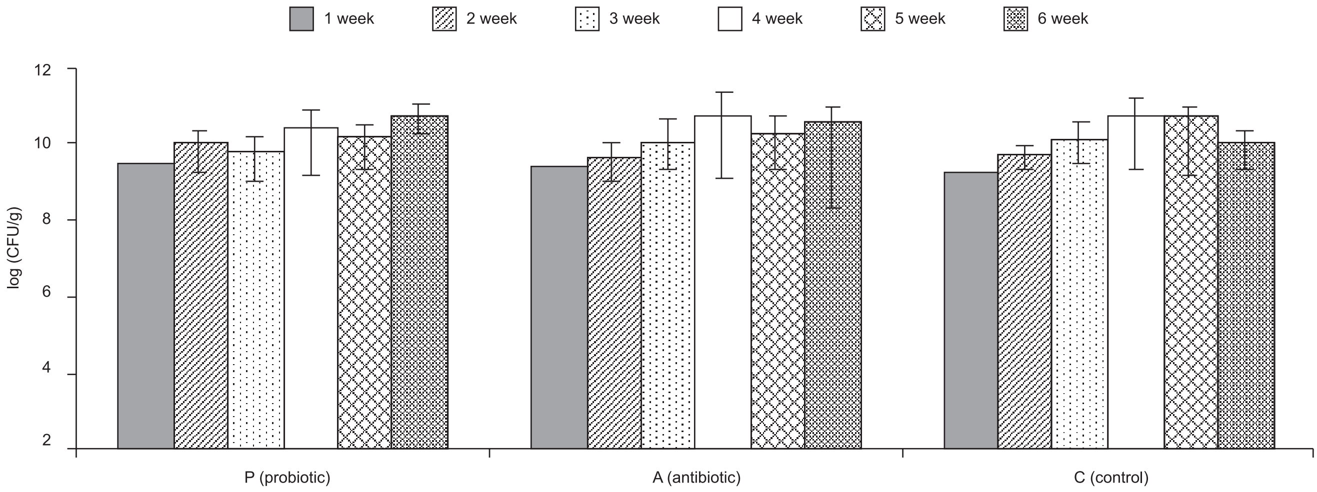


FIGURE 1. Total number of bacteria in faeces of chickens.

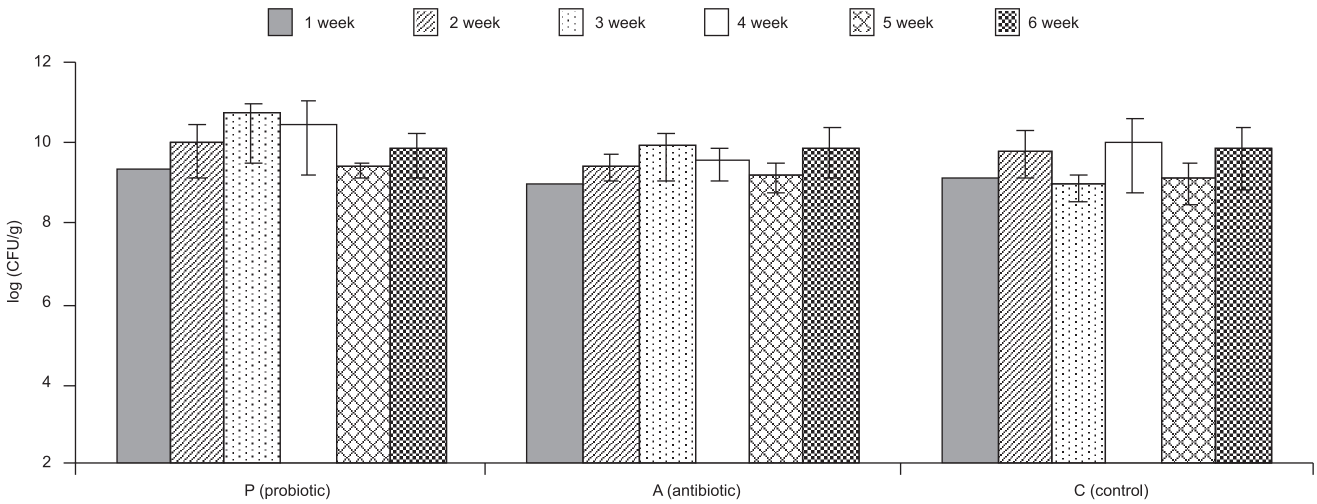


FIGURE 2. Bacteria of *Lactobacillus* in faeces of chickens.

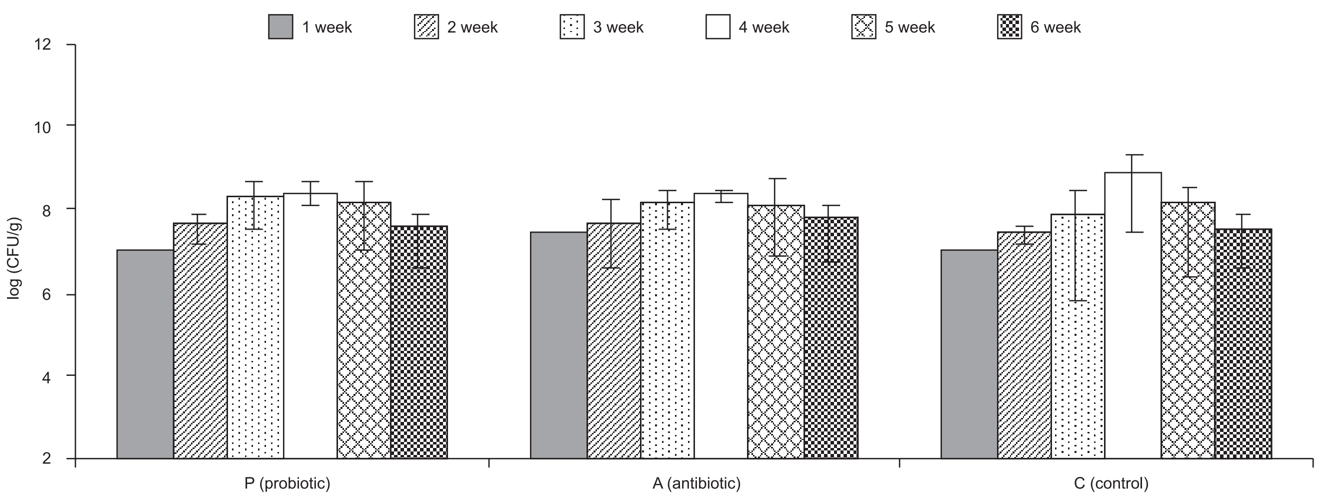


FIGURE 3. Bacteria of *Enterobacteriaceae* in faeces of chickens.

[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 *Coli* group bacteria in caecum in relation to the control sample, however only on 10 and 20 day of breeding. He did not find similar dependencies as for the small intestine. Corresponding research findings in this respect were presented by Francis *et al.* [1978]. Nevertheless, it should be emphasized that in the hereby research excreta of chickens fed with the feed mixed with a probiotic contained the lowest changeability of the number bacteria of *Enterobacteriaceae* family and bacteria belonging to *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 equaled *ca*. 10^5 CFU/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, irrespectively of the kind of feed supplementation, the researchers noted 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 (Figure 6). After the third week of breeding the further reduction in the number of the above-mentioned bacteria was observed only in the faeces of broilers fed with the feed containing a probiotic additive. The number equaled *ca*. 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, the researchers 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 the 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 it equaled *ca*. 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. The research outcome is particularly important as, since the antibiotic growth promoters

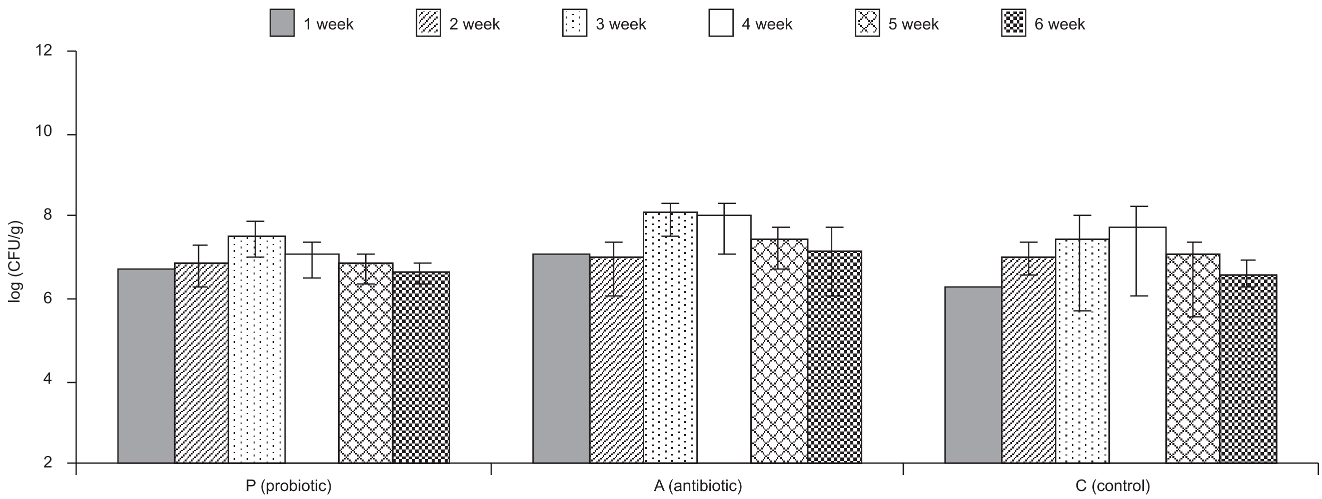


FIGURE 4. Total number of *Coli* group bacteria in faeces of chickens.

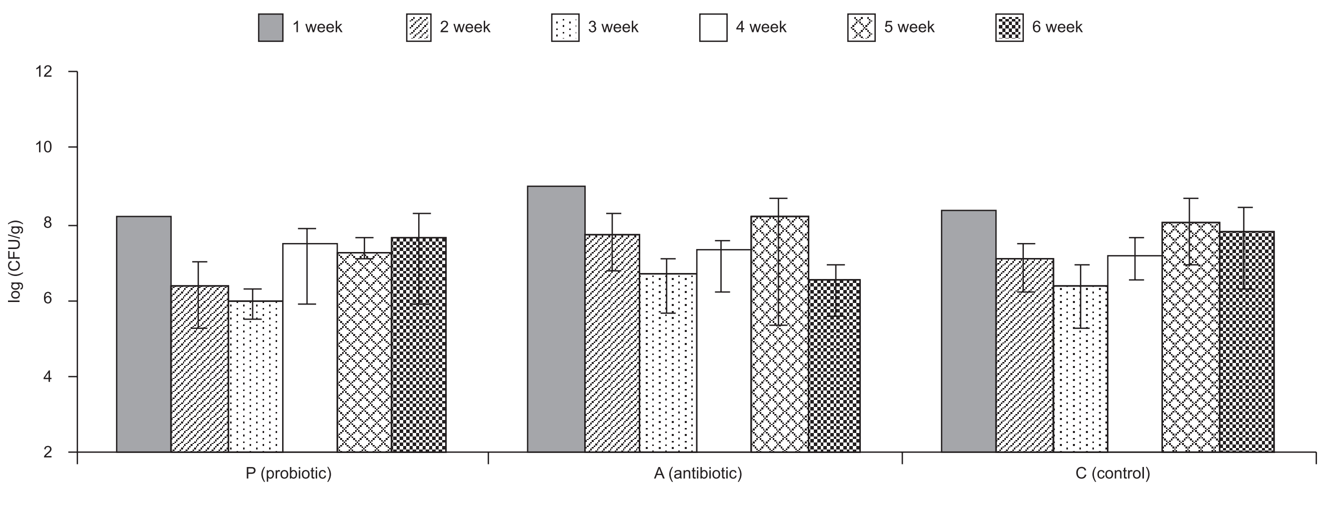
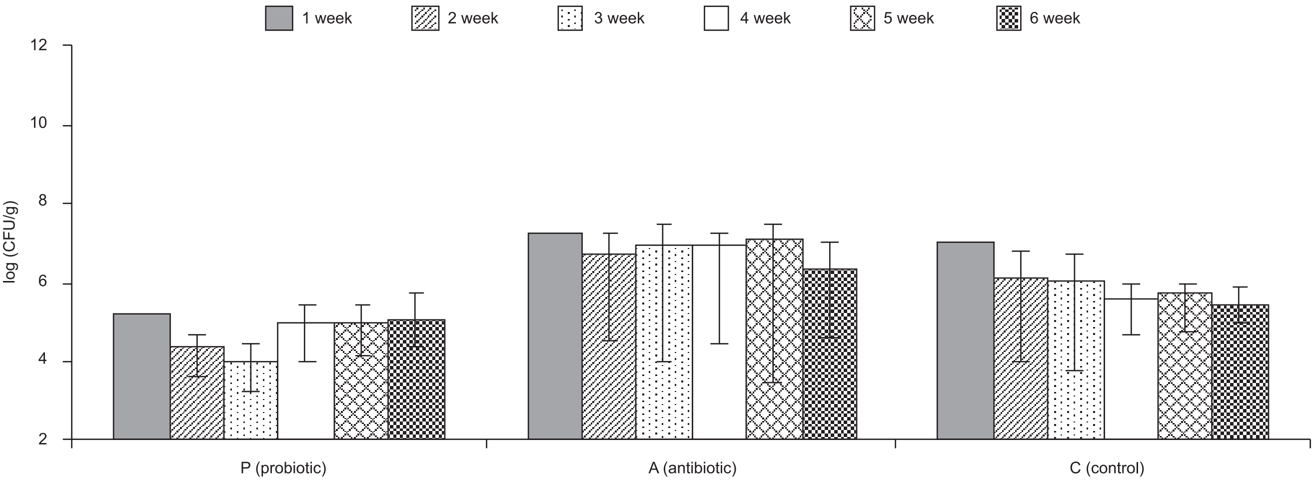


FIGURE 5. Bacteria of *Enterococcus* in faeces of chickens.



FIGURES 6. Bacteria of *Clostridium* in faeces of chickens.

were banned from the use in feeds for poultry, the number of intestinal problems in the case of such birds bred for consumption is likely to increase, especially as for problems 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 consequences were observed in other European countries.

CONCLUSIONS

On the basis of the hereby research 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. The designed technology of preparing a probiotic growth promoter was comprised in a patent, namely P-37136 "Probiotic growth promoter for poultry" – R. Zabielski, P. Michałowski, Z. Libudzisz, E. Klewicka, I. Motyl, K. Śliżewska, J. Biernasiak. The producer and distributor of the probiotic preparation called LABYuc-Probio® is Mifarmex Sp. z o.o. LABYuc-Probio® is admitted to trading on the Polish market and there are efforts being made to acquire admittance to trading in the Member States.

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WPŁYW PROBIOTYKU ZŁOŻONEGO Z BAKTERII *LACTOBACILLUS*, DROŻDŻY ORAZ EKSTRAKTU Z JUKI NA WYNIKI ODCHOWU I MIKROFLORĘ JELITOWĄ KURCZĄT BROJLERÓW

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Doświadczenie przeprowadzono na 99 kurkach brojlerach, podzielonych na 3 grupy po 33 ptaki, utrzymywane indywidualnie. Dieta pszenno-sojowa została podzielona na trzy części: P (z dodatkiem probiotyku, składającego się z bakterii kwasu mlekowego, drożdży i ekstraktu z juki), A (z dodatkiem flawomycyny) i C (nieuzupełniona). Diety skarmiano od 1 do 41 dnia życia. Końcowa masa ciała wynosiła we wszystkich grupach średnio 2,4 kg, przy użyciu 1,63 kg paszy/kg BWG. Dodatek do diety probiotyku bądź antybiotyku nie wpłynął na wydajność odchowu, masę wątroby, trzustki i przewodu pokarmowego. Uzupełnienie paszy preparatem probiotycznym już po pierwszym tygodniu odchowu wpłynęło na znaczną redukcję liczby bakterii z rodzaju *Clostridium* w kałomoczu kurcząt. Ponadto w kałomoczu kurcząt otrzymujących paszę z dodatkiem probiotyku odnotowano najniższą zmienność liczby bakterii z rodziny *Enterobacteriaceae* i bakterii z grupy *coli* w poszczególnych tygodniach odchowu.