Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 58 (1), 2019: 55–67
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 58 (1), 2019)
DOI 10.22630/AAS.2019.58.1.7

Comparison of the effect of lactic acid bacteria added to feed or water on growth performance, health status and gut microbiota of chickens broilers

MARTA KUPRYŚ-CARUK[1], MONIKA MICHALCZUK[2], BEATA CHABŁOWSKA[1], ILONA STEFAŃSKA[3]
[1]Institute of Agricultural and Food Biotechnology prof. W. Dąbrowski, Department of Fermentation Technology
[2]Faculty of Animal Science, Warsaw University of Life Sciences-SGGW
[3]Faculty of Veterinary Medicine, Warsaw University of Life Sciences-SGGW

Abstract: *Comparison of the effect of lactic acid bacteria added to feed or water on growth performance, health status and gut microbiota of chickens broilers.* The aim of the study was to evaluate the impact of two routs of potentially probiotic lactic acid bacteria (LAB) strains: *Lactobacillus plantarum* K KKP 593/p and *Lactobacillus rhamnosus* KKP 825 administration (via feed or water) on growth performance, health status and the composition of gut microbiota of broiler chickens. A total of 375 one-day-old chicks Ross 308 were divided into three main groups. The experimental factor was the application of bacteria to feed (starter, grower and finisher) or drinking water. Control group did not receive bacteria. Addition of bacteria to water had a favorable effect on higher live body weight of chickens during the first (starter) and the second (grower) period of rearing compared to the control group and the group receiving bacteria in feed. Total feed intake and feed conversion ratio was higher in the groups receiving bacteria than in the control group. Mortality among chickens receiving LAB was significantly reduced, wherein the lowest mortality was observed among chickens receiving bacteria in feed. Moreover, feeding chickens with potentially probiotic bacteria had an impact on inhibition the growth of *Clostridium perfringens* in the intestine and did not change biochemical and hematological parameters of blood and results of slaughter analysis compared to the control group. There were no clear and significant differences in analyzed parameters of chickens depending on the rout of lactic acid bacteria administration.

Key words: probiotics, chickens broilers, performance, blood parameters

INTRODUCTION

Nowadays the use of probiotics, as supplements added to feed, is currently a common practice in a large-scale rearing system of chickens broiler. Microorganisms, which are the most often used as probiotics, are lactic acid bacteria (LAB), as well as yeast (Ezema and Ugwu 2015, Hamidaa et al. 2015). It has been shown in many recent studies, that probiotics reduce chickens' mortality, improve the dressing percentage, stabilize the microbial composition of gastrointestinal tract (Brzóska et al. 2007, Awad et al. 2010). What is more, probiotics enhance the maintenance and function of the epithelial barrier and improve intestinal nutrient absorption, which is in association with the intestinal architecture improvement (Jahromi et al. 2015).

On the other hand, there are many reports describing the lack of influence of probiotics on weight gain or feed conversion of chickens broiler (Olnood

et al. 2015a). Brzóska et al. (2012) observed the beneficial effect of *Lactobacillus* spp. or *Lactococcus lactisstrains* on chickens' livability, but no effect on body weight gain and feed conversion. Lack of effectiveness of probiotics used as feed additives may be caused by many factors, such as variability of probiotics, probiotics species, level of consumption, duration and frequency of exposure to probiotics and physiological condition of the host, actual microbiota already present in the gut, methods of probiotic preparations production, as well as route of their administration (Brisbin et al. 2011, Huyghebaert et al. 2011, Ajuwon 2015).

Thera are a few routs of probiotics administration e.g. via feed, drinking water, by spraying on litter or by direct feeding to the beak (Olnood et al. 2015b). It has been shown that administration of probiotics via feed, compared to drinking water, contributed to a higher mean daily body weight gain of chickens (Timmerman et al. 2006).

The aim of the study was to evaluate if selected LAB strains have a positive effect on the growth performance and health condition of broilers and reducing undesired intestinal microflora counts depending on the method of their administration (via feed or drinking water).

MATERIALS AND METHODS

Birds and management

Broiler chickens ROSS 308 (not sexed) have been rearing for 42 days in the production hall in Agricultural Experimental Station Obory-Wilanów owned by Warsaw University of Life Sciences (SGGW) in Warsaw. A total of 375 one day old chicks, initially weighing 44.4 ±0.58 g, were divided into three main groups: two experimental groups F and W one control group C (in five replicates of 25 birds). Chickens were kept on straw litter in pens (at a stocking density of 11.4 birds per m^2). Air temperature, relative humidity and cooling conditions were controlled automatically and were the same for all the treatment groups. On the first day of age chicks were vaccinated against the following diseases: Marek's disease, Gumboro disease, infectious bronchitis and Newcastle disease. On the 15th day of age birds were vaccinated against bronchitis infectious.

Feeding program

During experimental period the broilers were fed according to the following feeding program: starter (1–21days), grower (21–35day), finisher (36–42 day) (*ad libitum* consumption). Feed for all experimental groups was made in mashed form from the same main compounds (Table 1).

Instarter, grower and finisher the coccidiostat Monteban® was also included. The experimental factor was the application of bacterial preparation to the feed or drinking water. Bacterial preparation was produced in the Department of Fermentation Technology in prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology in Warsaw and consisted of two potentially probiotic LAB strains: *Lactobacillus plantarum* K KKP 529/p and *Lactobacillus rhamnosus* KKP 825. The number of live bacteria in the preparation was 1.0×10^9cfu g^{-1}.

The bacterial preparation was added to feed during mixing of feed components at the feed plant. The capacity for ho-

TABLE 1. Composition of feed used in chickens broilers feeding program

Component (%)	Feed		
	Starter	Grower	Finisher
Maize meal	30.00	42.00	36.40
Wheatmeal	32.60	25.40	30.00
Soybean meal	28.60	21.60	17.80
Sunflower meal	3.00	5.00	5.00
Rapeseedmeal	–	–	5.00
Dicalcium phosphate	1.22	0.56	0.38
Sodium bicarbonate	0.14	0.10	0.14
Rapeseedoil	–	–	3.40
Sodium chloride	0.26	0.30	0.24
Fodderchalk	0.97	0.80	0.44

mogeneous distribution of the probiotic preparation in the final feed and drinking water was confirmed by tests performed according to instruction by Kwiatek and Przeniosło-Siwczyńska (2007) (data not presented).

Moreover, the stability of bacterial preparation (expressed in the number of live bacterial cells in 1 g of the feed) was confirmed under storage conditions of the final feed mixtures. Stability studies of the bacterial preparation in the final feed was of least six months' duration in accordance with the requirements described in Commission Regulation (EC) No 429/2008 of 25 April 2008. The stability of bacterial preparation in drinking water was also confirmed by studies conducted under condition simulating practical use (data not presented).
Experimental design looked as follows:
C: control group (not receiving bacteria)
F: group receiving bacteria in feed

W: group receiving bacteria in drinking water

Water was changed once a day. The quantities of active agents in the preparation (live lactic acid bacteria) introduced to the diet and expected daily intake of bacteria are presented in Table 2. Amounts of preparation added to feed and water were calculated to obtain the assumed concentration of bacteria and were based on previously obtained data on daily intake of feed and water by chickens under the same experimental conditions.

Observations

Chickens were individually weighted at the age of 1, 21, 35 and 42 days. After 21, 35 and 42 days of rearing, the total feed consumption of particular diet was determined for each group. Mortality was monitored during the whole rearing period.

At the end of rearing (42 day) 12 chickens (6 males and 6 females),with a body weight close to the average weight in a given group, were taken from each group for slaughter. Blood was taken from the wing vein. A whole small intestine was sampled for microbial analysis. On the following day the carcasses were

TABLE 2. Calculated quantities of live bacteria in feed and drinking water and suspected daily intake of bacteria by a chicken

Week	Amount of preparation in feed (g kg^{-1})	Number of bacteria in feed (CFU g^{-1})	Daily intake of bacteria via feed (CFU $piece^{-1}$ day^{1})	Amount of preparation in water (g L^{-1})	Number of bacteria in water (CFUL^{-1})	Daily intake of bacteria via water (CFU $piece^{-1}$ day^{1})
1	0.50	5.0×10^5	1.8×10^7	0.3	3.0×10^8	$0,7 \times 10^7$
2	0.50	5.0×10^5	1.8×10^7	0.3	3.0×10^8	1.9×10^7
3	0.15	1.5×10^5	1.8×10^7	0.1	1.0×10^8	1.0×10^7
4	0.15	1.5×10^5	1.8×10^7	0.1	1.0×10^8	1.5×10^7
5	0.10	1.0×10^5	2.2×10^7	0.1	1.0×10^8	1.9×10^7

dissected to determine dressing percentage, the weight of breast muscle, leg muscles, abdominal fat, weight of stomach, liver and heart.

Microbiological analysis

After slaughtering the whole content of small intestine was collected, weighted, diluted 10 times with saline and then further diluted from 10^{-1} to 10^{-5}. From the last three dilutions 0.1 mL was plated on the appropriate medium for intestinal microbiota detection (in triplicate). Qualitative and quantitative microbiological analysis included the following indicators:

– *E. coli* – TBX Agar medium (Biorad), 42°C, deep culture,
– *Enterococcus* spp. – Slanetz-Bartley medium (Argenta), surface culture at. 37°C under microaerophilic conditions with the content of 5% O_2 in an incubator (Cellhouse Heto-holten) with the regulation of oxygen amount by using $CO_{2,}$
– *Lactobacillus* spp. – MRS medium (Oxoid), 37°C, deep culture,
– *Clostridium perfringens* – TSC medium (Oxoid), 37°C, deep culture, plates were flooded with a thin layer of agar,
– *Salmonella* spp. The presence of *Salmonella* was determined according to PN-EN ISO 6579: 2003 standard (valid during the studies). Initially the content of the intestine was diluted 10 times in Peptone water buffered (Merck) and preincubated for 20 h at 37°C. After incubation, cultured on Modified Semi-solid Rappaport Vassiliaclis Base (Oxoid) (applying by spotting 100 µl on the plates) and incubated for 24 h at 41°C. Positive samples were then transferred to Brillance Agar (Oxoid) (reducing inoculation). After reincubation onBrillance Agar for 20 h at 41°C and obtaining clean colonies, bacteria were identified using Api 20 E (BioMerieux).

Blood analysis

Blood samples (3mL) were taken into EDTA polystyrene tubes for hematological tests and delivered within two hours to a specialized veterinary diagnostic laboratory, where blood smear examinations and morphological analyses were performed (automatically).

Samples for biochemical analysis were centrifuged (20°C, 3000 rpm, 10 min). Biochemical parameters (aspartic aminotransferase AST, creatine kinase CK, bile acids, uric acid, total protein) in plasma were determined with the use of diagnostic kits (Pointe Scientific Polska).

Statistical methods

Statistical analysis consisted of calculating the mean values and standard deviations of tested parameters. The significance of differences mean values was determined by Anova test and post-hoc Tukey test or (in the absence of homogeneity of variance) Anova rank Kruskal--Wallis test and multiple comparisons (test with Bonferroni correction). The level of significance was reported at $P < 0.05$. Statistica 8.0 (Statsoft, Poland) program was used.

In order to check whether the mortality rate depended significantly on the feeding method (no probiotic, with the addition of probiotic to feed or water), a chi-square independence test was performed, assuming a significance level of 0.05. The analysis was carried out in Excel program.

RESULTS

Giving to broiler chickens the diets with potentially probiotic bacteria added to drinking water caused that the body weight of birds at 21 and 35 day of rearing increased significantly compared to un supplemented control group and was significantly higher than in the group receiving bacteria in feed. Final body weight at 42 day of rearing of chickens fed with bacteria was slightly higher (but not statistically significant) than in the control group and there were no significant differences in the final body weight of chickens depending on the route of bacteria administration (Table 3).

Feeding chickens with potentially probiotic bacteria resulted in much lower mortality compared to the control group. The lowest value of mortality was observed in the group of chickens receiving bacteria in feed (Table 3).

Feeding chickens with potentially probiotic bacteria had an impact on to-

TABLE 3. Growth performance, mortality and feed conversion ratio depending on the route of bacteria administration

Item	Groups		
	C	F	W
Body weight at 21. day (g)	787.8^{a} ±100.2	796.5^{b}±109.2	809.9^{ab} ±116.9
Body weight at 35. day (g)	1888.9^{a} ±313.9	1923.6^{b}±258.1	2010.7^{ab}±273.3
Body weight at 42. day (g)	2555.0^{ab} ±367.8	2643.0^{a}±293.4	2688.3^{b} ±359.5
Mortality (%)	3.2^{ab}	0.8^{ac}	1.6^{bc}
Total feed intake (kg)	4.27^{ab} ±0.255	4.61^{a}±0.168	4.72^{b} ±0.065
Feed conversion ratio (kg kg^{-1} BW)	1.67^{ab}±0.052	1.74^{a}±0.065	1.76^{b} ±0.063

BW – body weight; a, b – mean values marked by the same letters (in rows) differ significantly ($P \leq 0.05$); ±standard deviation

tal feed intake and feed conversion ratio. These parameters were higher in the groups receiving bacteria than in the control group but without significant differences between groups receiving bacteria in feed or water (Table 3).

There were no significant differences in the proportion of particular determinants of slaughter analysis between males from all experimental groups, as well as between females. There were no differences in slaughter analysis of males or females depending on the route of bacteria administration ($P \geq 0.05$) (Table 4).

Some authors claimed that values of birds' blood parameters depend on sex (Kaceci and Col 2011, Abdi-Hachesoo et al. 2013). For that reason differences between particular parameters were analyzed separately for males and females.

In this study there were no significant differences between particular biochemical and hematological parameters among experimental groups in relation to males and females ($P \geq 0.05$) (Table 5).

The significant influence of potentially probiotic bacteria added to the diets were observed in the case of the number of *Escherichia coli* in the gastrointestinal tract of chickens. Giving bacteria via feed resulted in the lowest number of *E. coli* compared to the rest of experimental groups. Moreover, in the groups receiving bacteria via feed or drinking water, *Clostridium perfringens* was not detected. There were no differences between the number of *Enterococcus* spp. and *Lactobacillus* spp. among all groups ($P > 0.05$). Bacteria added to feed or water did not inhibit the growth of *Salmonella* spp. in the gastrointestinal tract

TABLE 4. Slaughter analysis of chickens broilers

Item	Sex	Groups		
		C	F	W
Dressing percentage (%)	M	73.0 ±1.44	72.7 ±1.81	72.4 ±3.76
	F	73.2 ±1.85	72.8 ±1.84	72.9 ±1.80
Breast muscle (% LBW)	M	31.4 ±1.53	29.9 ±1.71	30.9 ±3.01
	F	30.7±2.05	30.8 ±1.49	29.7 ±1.77
Leg muscle (% LBW)	M	20.6 ±2.45	19.7 ±1.89	19.5 ±1.80
	F	18.7 ±2.66	20.3 ±0.83	20.3 ±0.78
Abdominal fat (% CAR)	M	0.6 ±0.13	0.6 ±0.20	0.4 ±0.18
	F	0.6 ±0.14	0.8 ±0.34	0.7 ±0.25
Gizzard (% CAR)	M	1.1 ±0.14	1.1 ±0.16	1.1 ±0.15
	F	1.1 ±0.12	1.1 ±0.12	1.1 ±0.14
Liver (% CAR)	M	1.8 ±0.21	1.7 ±0.17	1.6 ±0.10
	F	1.9 ±0.27	1.8 ±0.15	1.8 ±0.34
Heart (% CAR)	M	0.5 ±0.05	0.4 ±0.03	0.4 ±0.04
	F	0.4 ±0.04	0.4 ±0.04	0.4 ±0.04

M – male, F – female, CAR – carcass, LBW – live body weight, ±standard deviation

TABLE 5. Biochemical and hematological blood parameters of chickens broilers

Item	Sex	Groups		
		C	F	W
AST (U L^{-1})	M	382.0 ±61.8	472.0 ±138.5	424. 7 ±136.5
	F	405.2 ±46.1	524.5 ±75.5	477.5 ±103.0
CK (U L^{-1})	M	9182.4 ±1700.2	10307 ±1543.0	8566.0 ±867.8
	F	9479.4 ±2085.8	11086.5 ±867.8	10772.3 ±1260.7
Total protein (G L^{-1})	M	24.6 ±3.30	26.4 ±0.89	26.8 ±1.50
	F	27.0 ±1.41	24.6 ±2.07	28.4 ±3.65
Uric acid (Mg dL^{-1})	M	2.5 ±0.92	2.4 ±0.60	2.5 ±0.62
	F	2.9 ±1.11	3.5 ±2.13	3.0 ±0.93
Bile acids (µmol L^{-1})	M	11.0 ±6.12	6.30 ±2.41	10.85 ±3.11
	F	6.86 ±4.92	4.18 ±1.15	10.84 ±5.21
Total RBC(T L^{-1})	M	3.0 ±0.52	2.4 ±0.03	2.8 ±0.36
	F	2.5 ±0.15	2.8 ±0.34	2.6 ±0.12
Lymphocyte (%)	M	22.0 ±9.17	43.7 ±22.30	32.7 ±12.22
	F	43.0 ±0.86	37.3 ±7.03	52.7 ±11.50
Hematocrit PCV (%)	M	33.4 ±5.50	26.9 ±1.05	30.5 ±3.93
	F	27.4 ±6.55	30.1 ±2.88	28.8 ±1.39
Hemoglobin (g dL^{-1})	M	13.6 ±2.24	11.4 ±0.35	13.1 ±1.76
	F	11.5 ±1.72	13.1 ±1.61	12.3 ±0.66

M – male, F – female , ± standard deviation

of chickens (*Salmonella enterica* subsp. *Arizoneae* was identified), while the presence of *Salmonella* spp. was not marked in litter and feces (the test for *Salmonella* spp. in litter and feces was performed by a veterinarian, who took care of the flock while experimental trial) (Table 6).

DISCUSSION

In this study potentially probiotic bacteria influenced on growth performance of broiler chickens during the first and the second period of rearing (starter and grower feed mixtures), wherein administration of bacteria via drinking water had more beneficial effect on the birds' growth than administration bacteria via feed.

In another work the authors did not observe statistically significant changes in the growth of sheep receiving probiotic in the initial period of administration compared to controls. The difference was marked only after 120 days of probiotic use (Lopez et al. 2012). The beneficial effect of probiotics on performance parameters was observed in studies conducted on turkeys (Lipiński et al. 2011), as well as on chickens broilers in study of Sabatkova et al. (2008), who showed that probiotic increased the final body weight and digestibility of feed by

TABLE 6. The effects of lactic acid bacteria routes of administration (via feed or water) on chickens' intestinal microbiota (log CFU g^{-1})

Item	C	F	W
Escherichia coli	8.95^{a}	7.85^{ab}	8.65^{b}
Clostridium perfringens	4.34	ND	ND
Enterococcus spp.	6.58	6.86	6.86
Salmonella spp.	+	+	+
Lactobacillus spp.	8.95	8.79	8.65

+ present, ND not detected, a, b –mean values marked by the same letters (in rows) differ significantly ($P \leq 0.05$)

4–5%. This is an evidence that the effect of probiotics is variable and uneven and its manifestation can occur after a long period of application.

Clinical signs of illness in birds are frequently subtle, so blood chemistry is necessary to evaluate cellular changes (Simaraks et al. 2004). Because of the fact, that hematological or biochemical parameters are influenced by diet, age, rearing behavior, environmental conditions, bird species and sex, it is difficult to compare the results of birds' blood parameters with results of other authors (Kaceci and Col 2011, Albokhadaim et al. 2012). In this study AST and CK activities were very high and ranges from 382 to 524 and from 8566 to 11086 U L^{-1} respectively. Specially AST activity was much higher than maximum reference value (Mazurkiewicz 2015) and also higher than in other described studies (Arslan et al. 2001, Albokhadaim et al. 2012). AST activity is a nonspecific biomarker of hepatocellular disease and is used with the muscle-specific enzyme creatine kinase (CK) to differentiate between liver and muscle damage (Harr 2002, 2005). High values of AST and CK activities can be explained by the fact, that these values increase in birds' blood under stressful circumstances (e.g. during slaughtering), which could not be completely eliminated under experimental conditions.

In this study total protein in blood of chickens from all experimental groups was consistent with the reference values reported in the literature (Clinical Diagnostic Division 1990, Mazurkiewicz 2015), so no nephropathy, enteropathy, liver failure or dehydration was observed, which could be diagnosed by lower or higher total protein values (Harr 2005). Renal disease was not observed as evidenced by the concentration of uric acid in blood of chickens from all experimental groups, which was in accordance with references values (Clinical Diagnostic Division 1990, Mazurkiewicz 2015). In the study of Albokhadaim et al. (2012) the concentration of uric acid in the blood of four-week-old chickens was higher than in this study but chickens were rearing at much higher temperature (32°C).

Feeding chickens with potentially probiotic bacteria did not cause liver damage as evidenced by the concentration of bile acids lower that 75 μmol L^{1}. The value higher than 75 μmol L^{1} suggests hepatic insufficiency, while higher

than 100 μmol L^{-1} is diagnostic for decreased liver function (Harr 2005). This is an evidence, that bacteria added to feed or water do not constitute environmental hazards influencing on liver function.

In this study LAB added to feed or water did not influence on the physiological condition of chickens broilers, which manifested by no differences in hematological parameters among all experimental groups of birds. Marked parameters were within the standard range of values (Clinical Diagnostic Division 1990, Mazurkiewicz 2015).

In literature little information is available about the impact of probiotics on hematological parameters of chickens. For instance, chickens broiler (males) were fed with probiotic (*Enterococcus faecium*) and no significant differences were observed in the number of erythrocytes and lymphocytes between chickens receiving and not receiving probiotics (Lan et al. 2017). In another study one-time administration of probiotic to one-day-old chicks resulted in increasing concentration of blood-carbohydrate, glucose, protein and hemoglobin levels after 35 days of rearing (Seifi et al. 2017).

The ability of *Lactobacillus* spp. to reduce the growth of undesirable microorganisms in the gastrointestinal tract of chickens broiler has so far been confirmed in many previous studies. For instance, *L. johnsonii* strain was used in drinking water, feed, sprayed on litter or administered directly to the beak, during 21 days of Cobb broiler rearing. The number of *Clostridium perfringens* and *Enterobacteriaceae* in the small intestine was reduced regardless of the method of probiotics administration (Olnood et al. 2015b).In subsequent studies probiotic bacteria (*L. johnsonii*) added to feed reduced the number of *Clostridium perfringens* and *Salmonella sofia* in the gastrointestinal tract of birds compared to the control group not receiving probiotics (Olnood et al. 2015c). In this study bacteria added to feed or water inhibited the growth of *Clostridium perfringens* but did not inhibit the growth of *Salmonella* spp. in the intestine, however, the strains used in this trial inhibited the growth of pathogenic microflora *in vitro* studies (Kupryś-Caruk et al. 2017). What is important, in this study *Salmonella* spp. was not detected in the litter and excreta of birds. *Salmonella* was detected in individual chickens. Propagation of *Salmonella* in the herd could not be large, hence the lack of pathogen's presence in litter or faeces, which samples were taken randomly from the whole production hall. This is an evidence, that no detection of *Salmonella* in litter or faeces does not provide that the herd is free from infection with this bacteria.

In this study it has not been clearly demonstrated which way of probiotics administration has more influence on selected performance parameters. Similar results were obtained by Brzóska and Stecka (2007), who did not observe significant differences in the body weight of chickens receiving bacteria (from *Lactobacillus paracasei* and *Lb. rhamnosus* genus) in feed and water. However, a tendency towards higher body weight of chickens receiving bacteria in water was observed. In the opinion of these authors, the efficiency of LAB is lower when used in dry feed mixtures compared to water, which may result from a relatively small amount of feed con-

sumed by chicks in the first days of their lives.

In trials of Giannenas et al. (2014) administration of probiotics through drinking water was no more effective than probiotics via feed. When comparing average daily weight gain, it can be observed that administration of probiotics via drinking water (Timmerman et al. 2006) generally resulted in a lower increase than administration via feed (Kalavathy et al. 2003). Giannenas et al. (2014) explained that this occurrence could be related to an adverse taste response of the water due to the organic acids present in the probiotic preparations. In Timmerman et al. (2006) study neutralization of the acids with calcium carbonate caused raising of water intake by chickens.

In the study of Karimi Torshizi et al. (2010) performance of broilers in terms of body weight gain, feed intake and feed conversion ratio were improved when probiotic was provided via drinking water, compared to the control and groups receiving probiotic in feed. What is more, spleen and bursa relative weights were influenced by the method of probiotic administration. Giving probiotic in water also improved T-cell dependent skin thickness response to phytohaemagglutinin (PHA) injection. The authors concluded, that the method of probiotic administration can influence the performance and immune competence of birds, and administration via drinking water appears to be superior to supplementation method via feed. More positive effect of probiotics administered in water than in feed was also obtained by Eckert et. al. (2010). The authors observed, that probiotics, when administered intermittently in the drinking water immediately after placement, followed by dietary feed changes the day before, the day of, and the day after placement, have the potential to increase body weight and decrease mortality-corrected FCR in broilers. The authors concluded, that for the product evaluated, post-pelleting feed application may not be as efficacious as drinking water application as a route of probiotic administration, when using performance parameters as indicators.

Lower than expected efficiency of probiotics administered via feed or water may result from instability of probiotic feed additive. Probiotics should be capable of exerting beneficial effects on the host and, what is more, be stable in storage and field conditions (Ezema and Ugwu 2015). According to Commission Regulation (EC) No 429/2008 for feed additives which consist of probiotic microorganisms (active agents) stability is defined in terms of loss of bacteria viability. In this context viability of bacteria can be limited in pelleted mixtures, because during pelleting process or other forms of treatment, bacteria are exposed to elevated temperature and pressure. At current industry practices, temperatures used in some feed mills may even reach 90°C, what is used for controlling feed pathogens such as *Salmonella* (Doyle and Erickson 2006). For that reason probiotics, as live microorganisms, should be able to tolerate harsh feed processing practices. In the study of Amerah et al. (2013) a probiotic supplementation based on tolerant on high temperatures *Bacillus subtilis* strains was used in nutritional experiment. Probiotic supplementation reduced feed intake by 2.1% and improved feed conversion ratio by

2.3% at 42 day, regardless of pelleting temperature.

The lack of unambiguous effectiveness of the probiotic administered via the feed may be caused also by inhomogeneous distribution of the feed additive in pre mixtures or other feeding stuffs, which is revealed by uneven intake of the probiotic by animals.

CONCLUSION

The role of probiotic bacteria added to chickens' diets is primarily the stabilization of the microbiota in the digestive tract, which further contributes to the improvement of health status and reduction mortality of birds. In this study mentioned relationship was observed with the simultaneous lack of the influence of bacteria administration on the final growth performance regardless of the route of bacteria administration. On the basis of the results obtained, one can induce the statement that in the case of the application the LAB strains, the effectiveness of their use is not dependent on the route of administration (via feed or water).

REFERENCES

ABDI-HACHESOO B., TALEBI A., ASRI-REZAEI S., BASAKI M. 2013: Sex related differences in biochemical and hematological parameters of adult indigenous chickens in northwest of Iran. J. Anim. Sci. Adv. 3(10): 512–516.

AJUWON K.M. 2015: Toward a better understanding of mechanisms of probiotics and prebiotics action in poultry species. J. Appl. Poult. Res. 25: 277–283.

ALBOKHADAIM I., ALTHNAINAN T., EL-BAHR S. 2012: Investigation of selected biochemical parameters of local chickens with different age and sex in Al-ahsa, Saudi Arabia. PJBS 15(17): 827–832.

AMERAH A., QUILES A., MEDEL P., SANCHEZ J., LEHTINEN M., GRACIA M. 2013: Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. Anim. Feed Sci. Tech. 180: 55–63.

ARSLAN M., OZCAN M., MATUR E., COTELIOGLIU U., ERGUL E. 2001: The effect of vitamin E on some blood parameters in broilers. Turk. J. Vet. Anim. Sci.25: 711–716.

AWAD W.A., GHAREEB K., ABDEL-RAHEEM S., BOHM J. 2010: Effect of addition of a probiotic micro-organism to broiler diet on intestinal mucosal architecture and electrophysiological parameters. J. Anim. Physiol. Anim. Nutr. 94: 486–494.

BRISBIN J.T., GONG J., OROUJI S., ESUFALI J., MALLICK A.I., PARVIZI P., SHEWEN P.E., SHARIF S. 2011: Oral treatment of chickens with lactobacilli influences elicitation of immune responses. Clinic. Vacc. Immunol. 18: 1447–1455.

BRZÓSKA F., BULUCHEVSKIJ S., STECKA K., ŚLIWIŃSKI B. 2007: The effects of lactic acid bacteria and mannan oligosaccharide, with or without fumaric acid, on chicken performance, slaughter yield and digestive tract microflora. J. Anim. Feed Sci. 16: 241–251.

BRZÓSKA F., STECKA K. 2007: Effect of probiotic, prebiotic and acidifier on the body weight of broiler chickens, feed conversion, and carcass and meat composition. Ann. Anim. Sci. 7: 279–288.

BRZÓSKA F., ŚLIWIŃSKI B., STECKA K. 2012: Effect of *Lactococcus lactis* vs. *Lactobacillus* spp. bacteria on chicken body weight, mortality, feed conversion and carcass quality. Ann. Anim. Sci. 12: 549–559.

CLINICAL DIAGNOSTIC DIVISION 1990: Veterinary reference guide: A sum-

mary of reference intervals for use with KODAK EKTACHEM products, Rochester (NY): Eastman Kodak Company

DOYLE M., ERICKSON M. 2006: Reducing the carriage of foodborne pathogens in livestock and poultry. Poult. Sci. 85: 960–973.

ECKERT N., HYATT J., STEVENS S., ANDERSON S., ANDERSON P., BELTRAN R., SCHATZMAYR G., MOHNL M., CALDWELL D. 2010: Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and nonmedicated diets. J. Appl. Poult. Res. 19(1): 59–67.

EZEMA C., UGWU C. 2015: Yeast (*Saccharomyces cerevisiae*) as a probiotic of choice for broiler production. In: Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas. Microbiology Monographs 29, Springer, 59–79.

GIANNENAS I., TSALIE E., TRIANTAFILLOU E., HESSENBERGER S., TEICHMANN K., MOHNL M., TONTIS D. 2014: Assessment of probiotics supplementation via feed or water on the growth performance, intestinal morphology and microflora of chickens after experimental infection with *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*. Avian Pathol. 43(3): 209–216.

HAMIDAA F, WIRYAWANB K, MERYANDINI A. 2015: Selection of lactic acid bacteria as probiotic candidate for chicken. Media Peternakan 38(2): 138–144.

HARR K. 2002: Clinical chemistry of companion avian species: A review. Vet. Clin. Path. 31: 140–151.

HARR K. 2005: Diagnostic value of biochemistry. In: Clinical avian medicine 2. Spix Publishing, 612–630.

HUYGHEBAERT G., DUCATELLE R., VAN IMMERSEEL F. 2011: An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187: 182––188.

JAHROMI M., ALTAHER Y., SHOKRYAZDAN P., EBRAHIMI R., IDRUS Z., TUFARELLI V., LIANG J. 2015: Dietary supplementation of a mixture of *Lactobacillus* strains enhances performance of broiler chickens raised under heat stress conditions. Int. J. Biometeorol. 66: 1099–1110.

KACECI T., COL R. 2011: Haematological and biochemical values of the blood of pheasants (*Phasianuscolchicus*) of different ages. Turk. J. Vet. Anim. Sci. 35(3):149–156.

KALAVATHY R., ABDULLAH N., JALALUDIN S., HO Y. 2003: Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br. Poultry Sci. 44: 139–144.

KARIMI TORSHIZI M., MOGHADDAM A., RAHIMI S., MOJGANI N. 2010: Assessing the effect of administering probiotics in water or as a feed supplement on broiler performance and immune response. Br. Poultry Sci. 51: 178–184.

KUPRYŚ-CARUK M., STEFAŃSKA I., CHABŁOWSKA B. 2017: Elimination of ochratoxin A by lactic acid bacteria strains isolated from chickens and their probiotic characteristics. Food Sci. Biotechnol. 81(2): 113–124.

KWIATEK K., PRZENIOSŁO-SIWCZYŃSKA M. 2007: Instrukcja badania homogeniczności pasz leczniczych i produktów pośrednich na podstawie badania stopnia wymieszania substancji czynnej. PIW, Puławy.

LAN R., LEE S., KIM I. 2017:Effects of *Enterococcus faecium* SLB 120 on growth performance, blood parameters, relative organ weight, breast muscle meat quality, excreta microbiota shedding, and noxious gas emission in broilers. Poultry Sci. 96 (9): 3246–3253.

LIPIŃSKI K., KALINIEWICZ J., TYWOŃCZUK J., STASIEWICZ M. 2011: Wpływ dodatków probiotycznych i ziołowych na produkcyjność i jakość mięsa indyków [The effect of probiotic and herbal additives on the productivity and meat quality of turkeys]. Rocz. Nauk. PTZ 7(2): 29–35 [in Polish].

LOPEZ Y., ARECE J., OJEDA F., ARÓSTICA N. 2012: Effect of the inclusion of the Sorbi fauna probiotic on lamb growth. Pastos y Forrajes 35(1): 109–118.

MAZURKIEWICZ M. 2015: Choroby drobiu. Wyd. AR, Wrocław [in Polish].

OLNOOD CH., BESKI S., CHOCT M.,IJI P. 2015a: Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim. Nutr. 1: 184–191.

OLNOOD CH., BESKI S., IJI P., CHOCT M. 2015b: Delivery routes for probiotics. Effects on broiler performance, intestinal morphology and gut microflora. Anim. Nutr. 1: 192–202.

OLNOOD C., BESKI S., CHOCT M., IJI P. 2015c: Use of *Lactobacillus johnsonii* in broilers challenger with *Salmonella sofia*. Anim. Nutr. 1: 203–212.

SABATKOVÁ J., KUMPRECHT I., ZOBAČ P., SUCHÝ P., ČERMÁK B. 2008: The probiotic BioPlus 2B as an alternative to antibiotics in diets for broiler chickens. Acta Vet. Brno 77: 569–574.

SEIFI K., TORSHIZI M., RAHIMI S., KAZEMIFARD M. 2017: Efficiency of early, single-dose probiotic administration methods on performance, small intestinal morphology, blood biochemistry, and immune response of Japanese quail. Poultry Sci. 96 (7): 2151–2158.

SIMARAKS S., CHINRASRI O., AENGWANICH S. 2004: Haematological, electrolyte and serum biochemical values of the Thai indigenous chicken (*Gallus domesticus*) in Notheastern Thailand. Song Klanakarin J. Sci. Technol. 26: 425–430.

TIMMERMAN H., VELDMAN A., VAN DEN ELSEN E., ROMBOUTS F., BEYNEN A. 2006: Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poultry Sci. 85: 1383–1388.

Streszczenie: *Wpływ bakterii kwasu mlekowego dodanych do paszy lub wody pitnej na wyniki produkcyjne, zdrowotność i mikroflorę przewodu pokarmowego kurcząt brojlerów.* Celem pracy była ocena wpływu dwóch potencjalnie probiotycznych szczepów bakterii kwasu mlekowego (LAB): *Lactobacillus plantarum* K KKP 593/p oraz *Lactobacillus rhamnosus* KKP 825 podawanych w paszy lub wodzie pitnej na wyniki odchowu, stan zdrowia i mikroflorę jelitową kurcząt brojlerów. 375 jednodniowych piskląt mieszańców Ross 308 podzielono na trzy główne grupy. Czynnikiem eksperymentalnym było zastosowanie preparatu probiotycznego w paszy (starter, grower i finiszer) lub wodzie pitnej. Grupa kontrolna nie otrzymywała preparatu. Dodanie preparatu probiotycznego do wody miało wpływ na większą masę ciała kurcząt po pierwszym oraz drugim okresie odchowu w porównaniu do grupy kontrolnej i grupy otrzymującej preparat probiotyczny w paszy. Całkowite spożycie i zużycie paszy było większe w grupach otrzymujących preparat w paszy lub wodzie niż w grupie kontrolnej. Śmiertelność wśród kurcząt otrzymujących LAB uległa istotnemu zmniejszeniu, przy czym najniższą śmiertelność zaobserwowano wśród kurcząt otrzymujących preparat probiotyczny w paszy. Ponadto, podawanie kurczętom probiotyku miało wpływ na zahamowanie wzrostu bakterii z gatunku *Clostridium perfringens* w jelicie oraz nie miało wpływu na cechy biochemiczne i hematologiczne krwi w porównaniu z grupą kontrolną. Nie stwierdzono wyraźnych i istotnych różnic w analizowanych cechach kurcząt w zależności od drogi podania bakterii kwasu mlekowego.

Słowa kluczowe: probiotyki, kurczęta brojlery, wyniki produkcyjne, parametry krwi

MS received 18.09.2018

MS accepted 15.02.2019

Authors' address:
Marta Kupryś-Caruk
Instytut Biotechnologii Przemysłu Rolno-Spożywczego im. prof. W. Dąbrowskiego
Zakład Technologii Fermentacji
ul. Rakowiecka 36
02-532 Warszawa
e-mail: marta.kuprys@ibprs.pl