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EFFECT OF *PEDIOCOCCUS* SPP. IN FEED INSTEAD OF ANTIBIOTIC ON BROILER CHICKEN BODY WEIGHT, MORTALITY, SLAUGHTER TRAITS AND MEAT QUALITY

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Abstract

In an experiment performed on 608 Ross 308 broiler chickens the effect of dietary *Pediococcus* Spp. 840 and Spp. 841 lactic acid bacteria (LAB) in the presence of mannan oligosaccharide and fumaric acid compared to the control unsupplemented group and the group supplemented with *Lactobacillus acidophilus* 842 (LAC) was studied. The body weight, feed intake, feed conversion, mortality, dressing percentage, proportion of valuable cuts in the carcass and physicochemical characteristics of breast muscle were investigated. Probiotic bacteria significantly reduced broiler mortality compared to the control group (P<0.01). No significant differences between feed intake and feed conversion were found. Feeding *Pediococcus* Spp. significantly increased chickens' slaughter weight and carcass weight (P<0.01). There were no significant differences in the weight and proportion of breast and leg muscles, and in the weight of gizzard, liver and depot fat. No significant differences occurred for the dry matter, protein and fat content of breast muscles. The same held true for blood plasma components and physical characteristics of muscle tissue such as hardness, springiness, cohesiveness, gumminess, chewiness and resilience. It was concluded that probiotic bacteria do not significantly modify the physicochemical and sensory parameters of breast muscles from broiler chickens.

Key words: lactic acid bacteria, broiler chickens, body weight, feed conversion, meat composition, physical characteristics of meat

Oligosaccharides, low-molecule organic acids and probiotic lactic acid bacteria have shown positive effects on reducing chicken mortality due to digestive tract infections, thus decreasing rearing costs (Patterson and Burkholder, 2003;

Brzóska and Stecka, 2007; Brzóska et al., 2007). Lactic acid bacteria act by colonizing different parts of the digestive tract, living in symbiosis with birds and stimulating their immune system (Dalloul et al., 2003; Huang et al., 2004; Koenen et al., 2004). Low-molecule organic acids acidify the digestive tract, which is paralleled by bacterial synthesis of lactic acid. Probiotic bacteria synthesize bacteriocins that inhibit pathogenic bacteria by reducing the incidence of infections causing proliferation of these bacteria, and by limiting the production of endotoxins that cause diarrhoea and mortality in birds (Cleveland et al., 2001). Probiotic bacteria are lactic acid bacteria belonging to the genera *Lactobacillus* Spp., *Enteroccocus* Spp., *Streptoccocus* Spp. and *Bacillus* Spp. Although many strains of lactic acid bacteria have been patented and put into production. Research is underway to obtain new bacteria characterized by more efficient synthesis of lactic acid and more efficient effect on broilers. To date, probiotic lactic acid bacteria were selected from diverse materials such as digestive contents of infants and animals or fermented dairy products. Most preparations contain *Lactobacilus* Spp. (including *L. acidophilus*) and *Enterococcus* Spp. bacteria. Several strains of *Pediococcus* Spp. with potential probiotic properties were obtained recently. These bacteria were assumed to show probiotic effects because of the rate of glucose metabolism. Several years' research on use of lactic acid bacteria in broiler chicken nutrition in our facility leads us to believe that the meat of chickens receiving probiotic bacteria has better taste compared to that of unsupplemented control chickens.

It is our hypothesis that probiotic bacteria may change some physical characteristics of poultry meat such as aroma, juiciness and tenderness, which results in better sensory score of muscle tissue.

The aim of this study was to determine if the *Pediococcus* Spp. probiotic bacteria fed to chickens can change their body weight, feed conversion, mortality, proportion of valuable cuts in the carcass, as well as palatability and physicochemical and sensory characteristics of meat.

Material and methods

A total of 608 Ross 1-day-old unsexed broiler chickens were randomly allotted to 4 groups, with 4 replications of 38 birds per group. Chickens were kept in cages with sawdust floors, stocking density was 18 birds/m^2 and 35 kg of birds/m^2 at the end of the trial. Broilers were fed maize, wheat and soybean meal diets to 42 days of age (Table 1). The nutrient content of feed was estimated by chemical analysis (AOAC, 1990). The control group received a diet without probiotic bacteria (Negative control) and a diet with *Lactobacillus acidophilus* 842 (Positive control). The experimental groups received diets supplemented with *Pediococcus* Spp. 841 and *Pediococcus* Spp. 840, respectively. All groups received oligosaccharides (BIOMOS; 1 g/kg feed) and fumaric acid (FUA; ORRFA-Polska Sp. z o.o.; 9.0 g/kg feed). The bacteria were used at a rate of 3 million bacterial cells/bird/day. LAB were added to drinking water. The probiotic bacteria originated from the collection of the Institute of Agricultural and Food Biotechnology in Warsaw.

Table 1. Components and nutritive value of the feeds

Item	Diet	
	Starter (1–21 days)	Grower (22–42 days)
Feed ingredients and supplements (%):		
Maize	34.00	30.00
Wheat	26.10	34.1
Soybean meal	32.50	28.50
Rapeseed oil	4.00	4.00
Dicalcium phosphate	1.70	1.70
Ground limestone	0.60	0.60
NaCl	0.35	0.35
L-lysine HCl (78%)	0.11	0.11
DL-methionine (99%)	0.14	0.14
Mineral-vitamin premix[1]	0.50	0.50
Nutrients in 1 kg dry matter:		
Crude protein (g)	219.7	208.5
Lysine (g)	12.9	11.5
Methionine+Cysteine (g)	4.8	4.2
Calcium (g)	8.8	8.3
Phosphorus (g)	4.2	4.1
Metabolizable energy (MJ)	12.44	12.23

[1]1 kg of starter diet contained: vit. A 13 5000 IU; vit. D_3 3 600 IU; mg: vit. E 45; vit. B_1 3.25; vit. B_2 7.5; vit. B_6 5; vit. B_{12} 0.0325; vit. K_3 3; biotin 0.15; nicotinic acid 45; calcium pantothenate 15; folic acid 1.5; choline chloride 100; Mn 100; Cu 1.75; Fe 76.5; Se 0.275; I 1; Zn 75; Co 0.4; Endox (antioxidant) 125; Sincox (coccidiostat) 1 g; calcium 0.679 g,

[1]1 kg of grower diet contained: vit. A 12 000 IU; vit. D_3 3 250 IU; mg: vit. E 40; vit. B_1 2; vit. B_2 7.25; vit. B_6 4.25; vit. B_{12} 0.03; vit. K_3 2.25; biotin 0.1; nicotinic acid 40; calcium pantothenate 12; folic acid 1.0; choline chloride 450; Mn 100; Cu 1.75; Fe 76.5; Se 0.275; I 1; Zn 75; Co 0.4; Endox (antioxidant) 125; Sincox (coccidiostat) 1 g; calcium 0.79 g.

Chickens were fed *ad libitum* with starter and grower diets (Table 1). Water was supplied via trough-type waterers. During the experiment mortality was recorded, feed intake was measured per pen, and average feed intake per bird was calculated. Chickens were weighed individually on days 21 and 42 of age, after 12 h fasting. On day 43 of age, 10 birds/group were randomly chosen (5 cockerels and 5 pullets) and slaughtered.

During slaughter, blood was collected into tubes containing small amounts of heparin and centrifuged, and plasma was frozen. After thawing, plasma was analysed for the concentration of glucose, total protein, triglycerides, total cholesterol and high-density lipoproteins (HDL) using Cormay diagnostic kits. Measurements were made with a Beckman DU 640 spectrophotometer.

After slaughter, hot carcasses, gizzard, liver, breast and leg muscles, and depot fat were weighed and the parts were stored in a room at +5°C for 24 h. On the following day, carcasses were dissected according to the procedure given by Zgłobica and Różycka (1972), and the weights of cold carcass, breast muscle and leg muscle were determined. Samples of the right breast muscle were minced and frozen at –18°C prior to subsequent chemical analysis. After thawing, the samples were analysed for dry matter, crude protein and crude fat. The analyses were performed using standard methods of the Central Laboratory of the National Research Institute of Animal Production, Poland, according to accepted procedures (AOAC, 1990). The second samples of breast muscle were collected for analysis of physical and sensory characteristics.

Twenty-four-hour cooling of carcasses was followed by sensory evaluation and instrumental measurement of muscle texture parameters. The force-deformation curve obtained served to calculate meat hardness, cohesiveness, springiness, chewiness and gumminess using a procedure described by Bourne (1982). Cylinder-shaped samples (14 mm in diameter and 15 mm in height) were cut from raw meat and from the meat roasted at 180°C to an internal temperature of 78°C. Shear force was measured using a TA-XT2 Texture Analyser (Stable Micro Systems) with a Warner-Bratzler attachment and a triangular notch in the blade. The blade speed during the test was 1.5 mm/s. The result was presented as force per area (kg/cm^2). Cylinder-shaped samples (14 mm in diameter and 15 mm in height) were cut from the meat roasted as above. The texture was analysed using the same texture analyser with an attachment in the form of a cylinder 50 mm in diameter. The samples were subjected to a double pressing test using a force of 10 g to 70% of their height. The cylinder speed was 2 mm/s, and the interval between pressures was 3 s. Breast muscle and leg muscle weight loss during heating (U_m) was calculated from the difference in muscle weight before and after heating and expressed in percent in relation to muscle weight before heating. At the end of sensory evaluations, the results were compared and arithmetic means were determined according to the PN-ISO 4121 standard. Mean scores for every quality characteristic were multiplied by severity coefficients appropriate for individual characteristics.

The results were analysed statistically by analysis of variance and Duncan's multiple range test, using Statgraphics software.

Results

Feeding probiotic bacteria to chickens significantly decreased their mortality compared to the control group (P<0.01). No significant differences were found in feed intake and feed conversion (Table 2). Feeding *Pediococcus* Spp. significantly increased slaughter weight and carcass weight (P<0.01). There were no significant differences in the dressing percentage, weight and proportion of breast muscles, leg muscles and weight of gizzard, liver and depot fat (Table 3). No significant differences occurred for the dry matter, protein and fat content of breast muscles and physiological parameters of blood plasma (Table 4).

Pediococcus Spp. probiotic bacteria in broiler diets had a statistically significant effect on pH of breast muscles 24 h after slaughter (Table 5). The highest value of pH 15 min postmortem was found for the muscles of broilers receiving a complete starter and grower diet supplemented with *Lactobacillus acidophilus* 842. Twenty-four hours postmortem, the highest pH value was characteristic of the muscles of control broilers receiving no probiotic bacteria. There were no statistically significant differences between texture parameters and shear force of muscles from different feeding groups of broilers. Although the meat of broilers fed a complete starter and grower diet with *Pediococcus* spp. 840 had higher texture parameters compared to broilers from the other feeding groups, there were no differences in meat appearance, colour, structure, aroma, juiciness and tenderness (Table 6). The meat of broilers receiving probiotic bacteria in their diets was characterized by better structure compared to control broilers (P<0.05). In addition, the meat of broilers receiving *Lactobacillus acidophilus* 842 in their diets was characterized by the most desirable taste compared to broilers from the other feeding groups (P<0.01). The application of severity coefficients showed that the meat of broilers receiving probiotic bacteria was of better quality compared to that of control broilers, although there were no significant differences between most quality parameters.

Table 2. Broiler chickens' body weight, mortality and feed conversion.

Item	Negative control*	Positive control**	Lactic acid bacteria		SEM
			Pediococcus Spp. 840	*Pediococcus* Spp. 841	
Body weight, 21 days (g)	831	849	831	839	8
Body weight, 42 days (g)	2590	2610	2582	2601	27
Mortality (%)	3.44 Aa	2.76 bB	2.20 cC	2.80 bB	0.30
Feed intake (kg/42 days)	4.45	4.45	4.46	4.52	0.22
Feed conversion (kg/kg BWG)	1.73	1.70	1.72	1.74	0.10

* – without lactic acid bacteria.
** – with Lactobacillus acidophilus 842.
a, b – values in rows with different letters differ significantly (P<0.05).
A, B – values in rows with different letters differ significantly (P<0.01).
SEM – standard error of the mean.
BWG – body weight gain.
CCW – cool carcass weight.
WCW – warm carcass weight.

Table 3. Slaughter weight, dressing percentage and carcass composition

Item	Negative control*	Positive control**	Lactic acid bacteria		SEM
			Pediococcus Spp. 840	*Pediococcus* Spp. 841	
Slaughter weight (g)	2580 aA	2550 aA	2601 bB	2591 bB	36
Carcass weight (g)	1980 aA	1962 aA	2000 bB	2041 bB	36
Dressing percentage (%):	76.7	76.86	76.92	78.76	4.99
breast muscles (CCW)	27.1	27.8	26.9	27.2	3.7
leg muscles (CCW)	20.9	20.9	21.1	21.5	4.2
gizzard (WCW)	1.6	1.6	1.4	1.4	0.1
liver (WCW)	2.3	2.2	2.3	2.3	0.3
abdominal fat (WCW)	2.4	2.9	3.3	2.5	0.2

a, b, A, B – values in rows with different letters differ significantly (P<0.05 and P<0.01).
For abbreviations see Table 2.

Table 4. Chemical composition of breast muscles and blood plasma parameters.

Item	Negative control*	Positive control**	Lactic acid bacteria		SEM
			Pediococcus Spp. 840	*Pediococcus* Spp. 841	
Chemical composition:					
Dry matter (%)	24.92	24.98	25.03	25.21	0.08
Crude protein (% DM)	23.57	23.90	23.75	23.73	0.08
Ether extract (% DM)	1.11	1.10	1.14	1.07	0.02
Plasma parameters (mg/dl):					
Glucose	255.0	268.9	265.8	261.0	2.7
Total protein	3.47	3.51	3.65	3.69	0.04
Triglycerides	36.66	34.33	36.09	37.34	0.78
Total cholesterol	137.4	140.4	144.6	146.7	2.0
High density lipoproteins	103.6	102.3	109.3	114.1	1.8
HDL/TCx100	75.4	72.8	75.6	77.8	

For abbreviations see Table 2.

Table 5. Physical parameters of chickens' breast muscles

Item	Negative control*	Positive control**	Lactic acid bacteria		SEM
			Pediococcus Spp. 840	*Pediococcus* Spp. 841	
Muscle pH after 15 min.	6.84 ab	6.94 b	6.85 ab	6.63 a	0.04
Muscle pH after 24 h	5.90 cC	5.85 bcBC	5.73 aA	5.77 abAB	0.01
Heat loss (%)	21.08 b	20.64 ab	18.39 ab	17.71 a	0.48
Shear force – fresh meat (kG/cm^2)	1.032	1.050	1.083	1.051	0.04
Shear force – roasted meat (kG/cm^2)	2.031	2.081	1.999	1.961	0.08
Hardness (N)	49.619	47.751	49.485	48.131	1.66
Springiness (cm)	0.457	0.464	0.516	0.493	0.01
Cohesiveness	0.419	0.425	0.425	0.438	0.01
Gumminess	20.31	19.74	23.32	20.01	0.74
Chewiness (N × cm)	9.06	9.56	12.46	10.56	0.62

a, b, c, A, B, C – values in rows with different letters differ significantly ($P<0.05$ and $P<0.01$).
For abbreviations see Table 2.

Table 6. Sensory quality of chickens' breast muscles (in points)

Palatability estimates	Negative control*	Positive control**	Lactic acid bacteria		SEM
			Pediococcus Spp. 840	*Pediococcus* Spp. 841	
Appearance	4.28	4.50	4.44	4.42	
Colour	4.19	4.51	4.45	4.49	0.34
Structure	3.91 a	4.39 b	4.16 ab	4.41 b	0.44
Aroma	4.01	4.15	3.88	3.98	0.06
Juiciness	3.70	3.97	3.97	3.83	0.05
Tenderness	3.90	4.26	4.17	4.05	0.15
Taste	3.67	3.85	3.72	3.65	0.40
Quality	good	good	good	good	0.24

a, b – values in rows with different letters differ significantly ($P<0.05$ and $P<0.01$).
For abbreviations see Table 2.

Discussion

The results of the present study with probiotic bacteria lead us to believe that not always and not all probiotic bacteria increase the body weight of broiler chickens. In other studies, *Lactobacillus paracasei* 824 and *Lactobacillus rhamnosus* 825 and 826 bacteria had a positive effect on chickens' body weight (Kalavathy et al., 2003; Jamroz et al., 2004; Brzóska et al., 2007), as confirmed by earlier research in this area (Brzóska et al., 1999 a, b). The positive effect of bacteria on the growth of broiler chickens was also observed by Simon et al. (2001) and Patterson and Burkholder (2003) for *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus* and *Streptococcus* bacteria. Gastrointestinal flora is an important protective barrier against pathogenic bacteria as it stimulates the development of birds' active immunity to pathogenic microorganisms (Perdigon et al., 1995). It has been demonstrated experimentally that *Enterococcus faecium* lactic acid bacteria inhibit the growth and development of *Salmonella pullorum* pathogenic bacteria, which considerably reduces bird mortality (Audisio et al., 2000). It was also shown that *Lactobacillus* bacteria increase immune resistance against infections with *Eimeria acervulina coccidia* (Dalloul et al., 2003). Finally it was shown that lactic acid probiotic bacteria are able to synthesize bacteriocins, known as active antibacterial substances in the digestive tract of birds (Joerger, 2003).

The available literature contains relatively little information about the probiotic activity of *Pediococcus* Spp. bacteria. The present study showed that probiotic bacteria have no significant effect on feed intake and feed conversion (kg feed/kg gain). This demonstrates that the presence of probiotic bacteria, a prebiotic, and low-molecule organic acids (fumaric acid) has no effect on the degree of nutrient digestion and absorption, as reflected in the level of plasma metabolic parameters.

The experiment described in this paper shows that two strains of *Pediococcus* bacteria, used as probiotic additives, reduce bird mortality during the rearing period while increasing slaughter weight and carcass weight in relation to the control group receiving no bacteria, and also in relation to the group receiving *Lactobacillus acidophilus* probiotic bacteria, which has been shown to be efficient in broiler nutrition by many earlier studies (Brzóska et al., 1999 a, b). From this it may be concluded that *Pediococcus* Spp. bacteria, similarly to *Lactobacillus* Spp. bacteria, metabolize glucose available in the intestinal mucosa to produce lactic acid while reducing and stabilizing gastrointestinal pH in a way that is optimal, thus preventing the growth of pathogenic bacteria that induce inflammations and gastritis resulting in diarrhoea and mortality. The beneficial effect of both bacteria on birds' body weight may suggest a positive effect on protein and polysaccharide hydrolysis and amino acid and glucose metabolism in chickens.

The type of probiotic bacteria given to chickens was found to have no effect on the proportion of different muscle parts in the carcass or on the weight of gizzard, liver and depot fat. This is in agreement with the results of many studies conducted earlier. The activity of probiotic bacteria is limited to the digestive tract, its efficient function, mobilization of the immune system, and thus enhancement of immunity to pathogenic bacteria found in feeds and in equipment in bird facilities. Probiotic bacteria given to birds did not have any effect on the formation and growth of different organs, especially the muscular system but also gizzard and liver, or on storage of energy reserves in the form of depot fat.

Probiotic bacteria had no influence on chemical composition of breast muscles. The dry matter, protein and fat content of muscles remained at a similar level. Likewise, no differences were observed in the level of serum physiological indicators, including glucose, total protein, triglycerides, and low density cholesterol. These data indicate that probiotic bacteria, including those of the genus *Pediococcus* Spp. that live in symbiosis in the gastrointestinal tract of birds do not disturb the basal metabolism not modify plasma metabolite levels.

Little information is available in the scientific literature concerning the effect of probiotic bacteria on poultry meat quality (Barteczko et al., 2003). Our findings, based on several years' study of probiotic bacteria in broiler nutrition indicate improved taste quality of broiler meat, including aroma and sensory quality determined conventionally by a panel of assessors. Because there are no analytical procedures to assess sensory quality of meat, we evaluated physical characteristics of breast muscle tissue. The chemical composition, texture parameters and sensory quality of poultry meat all depend on genetic factors (such as breed, genetic group and sex) and environmental factors (such as age, slaughter weight, feeding and environmental conditions). The sensory appeal of meat is determined predominantly by flavour and aroma, but an important role is also played by meat juiciness, tenderness, colour and general appearance. In a study by Połtowicz et al. (2006), roasted breast muscles of cockerels were characterized by lower chewiness scores, and the meat was softer but had greater shear force values. Compounds found in raw meat are regarded as precursors of flavour and aroma, but most of them become active during heat treatment. They are mostly fatty acid esters, which are released most intensively after cooking and especially during heat treatment, when frying and grilling poultry meat. Lyon et al. (2004) investigated the effect of diet on sensory and physical characteristics of breast muscles in broilers. The meat of maize-fed broilers was characterized by significantly higher lightness compared to that of wheat-fed broilers. Source of carbohydrates proved to have a measurable effect on the aroma and texture of broiler breast muscles (Lyon et al., 2004). According to Sosnówka-Czajka et al. (2005), thermal conditions and different levels of dietary energy have no important effect on the quality of broiler meat. Barteczko et al. (2006) showed that ground maize of different varieties had no significant effect on the sensory parameters of breast and leg muscles from broiler chickens, although it had a significant effect on shear force and hardness of broiler muscles.

The present results showed that *Pediococcus* Spp. probiotic bacteria differ significantly in their effect on muscle pH after 15 min and 24 h, and compared to birds receiving no probiotic bacteria, birds given these bacteria were characterized by lower loss of muscle tissue during cooking. For most parameters of muscle tissue studied, such as muscle fibre shear force, hardness, springiness, cohesiveness, gumminess, chewiness and resilience, differences between the groups were not significant. Our findings indicate that improvements in the taste characteristics of meat from broiler chickens receiving probiotic bacteria may be associated with their chemical composition and not with the physical parameters of the muscles. It is difficult to say whether the changes in chemical composition are associated with the metabolism of living organisms or with changes that occur in muscle tissue postmortem. It cannot be excluded that probiotic bacteria modify the processes that take place in muscle tissue protein and fat, which makes chicken meat more flavoursome.

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Wpływ bakterii *Pediococcus* Spp. w paszy zamiast antybiotyku, na masę ciała kurcząt, śmiertelność, wydajność rzeźną i jakość mięsa

STRESZCZENIE

W doświadczeniu wykonanym na 608 kurczętach broilerach ROSS 308 badano wpływ podawania w diecie bakterii *Pediococcus* Spp. 840 i Spp. 841 w obecności oligosacharydu mannanu i kwasu fumarowego, w porównaniu do grupy kontrolnej z *Lactobacillus acidophilus* 842 (LAC) oraz bez dodatków. Badano masę ciała, spożycie i wykorzystanie paszy, śmiertelność, wydajność rzeźną i udział cennych partii w tuszkach, a także cechy fizyko-chemiczne mięśnia piersiowego.

Podawanie kurczętom bakterii probiotycznych obniżyło istotnie śmiertelność kurcząt w porównaniu do grupy kontrolnej (P<0,01). Nie stwierdzono istotnych różnic w spożyciu i wykorzystaniu paszy. Podawanie bakterii *Pediococcus* Spp. istotnie zwiększyło masę ubojową kurcząt i masę tuszek (P<0,01). Nie stwierdzono istotnych różnic w masie i udziale mięśni piersiowych, mięśni nóg oraz masie żołądka, wątroby i tłuszczu zapasowego. Nie stwierdzono istotnych różnic w zawartości suchej masy, białka i tłuszczu w mięśniach piersiowych. Nie stwierdzono również istotnych różnic w składnikach osocza krwi, a także w cechach fizycznych tkanki mięsnej jak twardość, sprężystość, spoistość, gumowatość i żujność. Wnioskowano, że bakterie probiotyczne nie modyfikują istotnie mierzonych parametrów fizyko-chemicznych i sensorycznych mięśni piersiowych kurcząt rzeźnych.