EFFECT OF DIETARY PROBIOTIC *PEDIOCOCCUS ACIDILACTICI* MA 18/5 M AND PREBIOTIC MANNANOLIGOSACCHARIDES AND THEIR COMBINATION ON CAECAL PARAMETERS IN HENS

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Abstract. The aim of experiment was to investigate the physiological response of caecal ecosystem of laying hens to supplementation of a diet with probiotic or prebiotic preparations and with both of these additives. The experiment was conducted on 4 groups of laying hens, with 10 birds each, for 4 weeks fed standard diets with added probiotic preparation (Bactocell, containing *Pediococcus acidilactici*) and mannan-oligosaccharide (MOS), given as prebiotic preparation (Agrimos). The dietary treatments did not affect the analyzed caecal parameters: relative weight of tissue and digesta, dry matter concentration, and pH value of caecal digesta, but affected caecal ammonia level the activity of selected microbial enzyme. Dietary combination of Bactocell and MOS increased activity of α-glucosidase, α-galactosidase and β-galactosidase activity ($P<0.05$ *versus* other groups), and decreased β-glucuronidase activity (significantly in comparison to the control group). The lowest β-glucuronidase activity was observed upon a single MOS addition ($P<0.05$ *versus* control and probiotic treatments). Ammonia level was significantly lowered by mannan, as a single supplement and in combination as well ($P<0.05$ *versus* control and probiotic groups). As compared to the control group a single probiotic treatment increased concentration of acetate and total SCFA, while the applied combination of probiotic and MOS increased proportion of propionate, with simultaneously decrease in proportion of butyrate in the SCFA profile. It could be concluded that, along with the desired action in the caeca of hens as compared to the control birds, a dietary combination of applied probiotic strain with prebiotic MOS beneficially reduced caecal ammonia concentration and β-glucuronidase activity (*versus* single probiotic group) as well as increased propionate concentration (*versus* single prebiotic dietary treatment).

Keywords: probiotic, prebiotic, caecum, hens.

PROBIOTIKŲ *PEDIOCOCCUS ACIDILACTICI* MA 18/5 M, PREBIOTIKŲ MANANOLIGOSACHARIDŲ IR JŲ KOMBINACIJOS ĮTAKA DĖSLIŲJŲ VIŠTŲ AKLOSIOS ŽARNOS RODIKLIAMS

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Santrauka. Tyrimo tikslas – nustatyti dėsliųjų vištų aklosios žarnos ekosistemos fiziologinį atsaką į lesalų papildymą probiotikais, prebiotikais ir jų kombinaciją. Bandymas atliktas su 40 dėsliųjų vištų, suskirstytų į keturias grupes, kurios 4 savaites gavo lesalų su probiotiku „Bactocell“, sudarytu iš pieno rūgšties bakterijų *Pediococcus acidilactici,* ir prebiotiku „Garimos“, sudarytu iš mananoligosacharidų, bei minėtų probiotiko ir prebiotiko mišiniu. Lesalų priedai tirtų aklosios žarnos rodiklių – santykinio žarnos ir chimuso svorio, sausųjų medžiagų kiekio, pH vertės – neveikė, bet turėjo įtakos amoniako kiekiui ir mikrobinių enzimų aktyvumui. Lesalų papildymas „Bactocell“ ir „Garimos“ kombinacija padidino alfa gliukozidazės, alfa galaktozidazės ir beta galaktozidazės aktyvumą palyginti su kitomis grupėmis ($p<0,05$) ir statistiškai reikšmingai sumažino beta gliukuronidazės aktyvumą palyginti su kontroline grupe. Mažiausias beta gliukuronidazės aktyvumas nustatytas veikiant mananoligosacharidų priedui palyginti su kontroline ir probiotikus gavusia grupėmis ($p<0,05$). Amoniako kiekis statistiškai reikšmingai sumažėjo lesalus papildant tiek mananoligosacharidais atskirai, tiek kartu su *Pediococcus acidilactici* palyginti su kontroline ir tik probiotikus gavusia grupėmis ($p<0,05$). Palyginti su kontroline grupe dėl probiotikų priedo lesaluose padidėjo acto rūgšties ir bendras trumpųjų grandinių rūgščių kiekis, o dėl probiotikų ir MOS kombinacijos – propiono rūgšties kiekis. Galime daryti išvadą, kad palyginti su kontroline grupe didžiausią aklosios žarnos ekosistemos fiziologinį atsaką turėjo vištų lesalų papildymas *Pediococcus acidilactici* ir mananoligosacharidų kombinacija – sumažėjo amoniako koncentracija, beta gliukuronidazės aktyvumas ir padidėjo propiono rūgšties kiekis.

Raktažodžiai: probiotikai, prebiotikai, akloji žarna, dėsliosios vištos.

Introduction. The use of probiotic microorganisms, prebiotic substrates stimulating beneficial microbiota population, or symbiotic combinations of prebiotics and probiotics, has been reported as an alternative approach to dietary content of sub-therapeutic antibiotics in livestock feed (Patterson and Burkholder, 2003). Results of ample experiments indicate that probiotics could be successfully used as nutritional tools in poultry feed for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and finally better meat quality, however, these findings are still under thorough practical investigation (Kabir, 2009). A lack of documented physiological and microbiological effects of different probiotic preparations is an important reason for regulatory approval delays, particularly in Europe, regarding the commercial application of some microorganisms in poultry diets (Applegate et al., 2010). One possibility to increase the efficiency of dietary probiotics is simultaneous application of prebiotics, defined as non-digestible or low-digestible feed ingredients that benefit the host organism by selective stimulating the growth or activity of one or a limited number of probiotic bacteria in the gastrointestinal tract (Gibson and Roberfroid, 1995). The idea concerning a dietary combination of a probiotic with a prebiotic was supported by Rowland et al., (1998), who showed that the administration of probiotic inulin with the probiotic *Bifidobacterium longum* resulted an enhanced protective effect in the gut, compared to the administration of either probiotic or prebiotic separately. The results of some experiments on poultry (Biggs et al., 2007; Baurhoo et al., 2007) indicate that this role can perform mannanooligosaccharides (MOS). *In vitro* studies revealed a great potential of MOS to reduce attachment of enterobacteria and enhance adhesion of *Bifidobacterium* and selected *Lactobacillus strains* (Wasilewska et al., 2010). In earlier experiments *in vivo* it has been found, however, that the most commonly used dose of MOS (0.1%) did not affect the functioning of the intestinal ecosystem of turkeys (Zdunczyk et al., 2005). It was found, that probiotics and MOS had a positive effect on caecal metabolism of broiler chickens (Semaskaite et al., 2008). This indicates the need for further studies of the optimal use of MOS in poultry feeding, especially in combination with other non-antibiotic growth promoters.

The aim of this study was to examine the physiological response of gastrointestinal tract, especially caecal ecosystem of hens fed diet with probiotic preparation (containing *Pediococcus acidilactici* MA 18/5 M) and MOS, given separately or in combination.

Materials and methods. The procedures related to birds care used in this experiment followed the International Guiding Principles for Biomedical Research Involving Animals as Issues by the Council for the International Organizations of Medical Sciences and EU Directive 86/609/EEC and EC recommendations 2007/526 EC „Using and keeping of animals for experimental and other purposes“. The physiological study was conducted on 40 hens *Hisex Brown* cross at the age of 30 weeks allocated to four groups, each of 10 birds. The birds were kept individually and had free access to feed and tap water.

The hens were fed a standard diet without supplements (Control) or supplemented with a probiotic preparation (Bactocell, Lallemand Inc, Blagnac, France; 1×10^6 CFU/g lactic bacteria *Pediococcus acidilactici* MA 18/5 M), prebiotic preparation of mannan-oligosaccharides (MOS, Agrimos, Lallemand Inc, Blagnac, France; 2.5 kg/t of a diet) and with both supplements – Bactocell and MOS. All experimental diets were prepared using identical components whose nutritive value corresponded to the nutrient requirements of hens (NRC, 1994). The composition of the basal diet is given in Table 1.

Table 1. **Composition and nutritive value of a basal diet**

Ingredients	%	Nutrient composition	
Wheat	33.06	Metabolizable energy, MJ/kg	11.74
Barley	20.00	Crude protein*, %	17.41
Wheat meal	5.00	Crude fat*, %	4.87
Soya bean meal	16.00	Crude fiber, %	3.78
Corn	5.00	Crude ash, %	2.62
Sunflower meal	6.37	Ca*, %	3.88
Rapeseed oil	3.00	P*, % (total)	0.65
Salt	0.23	P, % (av.)	0.36
Limestone	9.50	Na , %	0.16
Monocalcium phosphate	1.00	Mg, %	0.30
Sodium bicarbonate	0.10	K, %	0.67
Vitamin-mineral premix	0.50	Cl, %	0.20
DL-methionine	0.22	NaCl, %	0.22
Santoquin	0.02	Lysine, %	0.74
		Methionine, %	0.44
		Methionine/Cysteine, %	0.76
		Tryptophan, %	0.21
		Threonine, %	0.59

* Analyzed values

At the end of the trial, the hens were killed by cervical dislocation according to the recommendations for euthanasia of experimental animals. The caeca with contents were taken from each bird, and as soon as possible after euthanasia (ca. 30 minutes), caecal pH was measured using a microelectrode and pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). Samples of caecal contents were used for immediate analysis: ammonia, dry matter, short-chain fatty acids (SCFA), while the rest of digesta was transferred to tubes and stored at -70°C. The caecal wall was flushed clean with ice-cold saline, blotted on filter paper, and weighed (caecal wall weight). Dry matter of caecal digesta was determined at 105ºC. In fresh caecal digesta samples, ammonia was extracted and trapped in a solution of boric acid in Conway dishes and was determined by direct titration with sulfuric acid. The bacterial glycolytic activity in the caecal digesta was measured by the rate of *ρ*- or *o*- nitrophenol release from their nitrophenylglucosides according to the modified method of Djouzi and Andrieux described by Juskiewicz et al. (2007). The following substrates were used: *ρ*-nitrophenyl-*α*-D-glucopyranoside (for *α*-glucosidase), and *ρ*-nitrophenyl-*β*-D-glucopyranoside (for *β*-glucosidase), *ρ*-nitrophenyl-*α*-D-galactopyranoside (*α*-galactosidase), *o*-nitrophenyl-*β*-D-galactopyranoside (*β*-galactosidase), and *ρ*-nitrophenyl-*β*-D-glucuronide (for *β*-glucuronidase). Caecal content was diluted (100 g/L) in 100 mM phosphate buffer (pH 7.0). The suspension was centrifuged for 15 minutes at 7,211 × g at room temperature, and enzymes were assayed on the supernatant. The reaction mixture contained 0.3 mL of a substrate solution (5 mM) and 0.2 mL of a dilution of the caecal sample. Incubation was carried out at 37ºC and *ρ*-nitrophenol was quantified at 400 nm and at 420 nm (*o*-nitrophenol concentration) after the addition of 2.5 mL of 0.25 M cold sodium carbonate. The enzymatic activity (*α*- and *β*-glucosidase, *α*- and *β*-galactosidase, and *β*-glucuronidase) was expressed as µmol product formed per hour per g of digesta. Caecal digesta samples were subjected to the SCFA analysis using gas chromatography (Shimadzu GC-2010; Shimadzu, Kyoto, Japan). The samples (0.2g) were mixed with 0.2 mL formic acid, diluted with deionized water and centrifuged at 7,211 × g for 10 min. The supernatant was loaded onto a capillary column (SGE BP21, 30 m × 0.53 mm) using an on-column injector. The initial oven temperature was 85ºC and was raised to180ºC by 8ºC /min and held there for 3 minutes. The temperatures of flame ionization detector and the injection port were 180 and 85ºC, respectively. The sample volume for GC analysis was 1 µL.

The results of the experiment were analyzed using the 1-way ANOVA test, and significant differences between groups were determined by Duncan's multiple range test. Statistica 8.0. for Windows™ software was used. Data in tables are given as means ± SEM. Differences were considered significant at $P \leq 0.05$.

Results. The mass of the caecal wall and digesta ranged within a narrow range, 2.78–2.89 and 3.89–4.21 g/kg body weight of hens, respectively (Table 2). Relatively small, statistically non significant differences between the groups were observed in the dry matter and pH of digesta. A significantly lower concentration of caecal ammonia was observed in laying hens fed diet containing MOS, as single supplement or when combined with Bactocell, in comparison to the control hens and the birds fed diet with probiotic single addition.

Table 2. **Caecal indices of broiler chickens**

	Experimental groups				PooledSEM	*P*
	Control	Probiotic	MOS	Probiotic+ MOS		
Tissue, g/kg BW	2.83	2.78	2.85	2.89	0.215	0.682
Digesta, g/kg BW	4.21	3.89	3.99	4.08	0.456	0.245
Dry matter, %	19.1	20.7	20.8	20.4	1.241	0.389
Ammonia, mg/g	0.46[a]	0.45[a]	0.30[b]	0.37[b]	0.049	0.037
pH	6.59	6.32	6.42	6.35	0.119	0.712

Values with the different letters differ significantly; ab – $P \leq 0.05$

There were significant differences in the activity of microbial enzymes studied (Table 3). Compared to the other groups, in the caeca of hens fed diets supplemented with probiotic and MOS higher activity of α-glucosidase ($P<0.001$), α-galactosidase ($P=0.001$) and β-galactosidase ($P=0.002$) was determined. Activity of β-glucosidase and β-glucuronidase was the highest in the control group and the lowest in hens fed diet containing MOS ($P=0.044$ and $P=0.009$, respectively).

The effects of dietary treatments on the short chain fatty acid concentration in the caeca of hens are summarized in Table 4. As compared with the control group, a statistically higher concentration of acetate was noted in the caecal digesta of hens fed a diet supplemented only with probiotic. In the groups fed diets containing MOS, both as a single supplement and in combination, the concentration of acetate in the caecal digesta was numerically, but not statistically, higher than in the control group. In the group fed diet containing probiotic and MOS the highest concentration of butyrate was determined, significantly higher than in the control group and the group fed a diet with MOS. Dietary application of probiotic and MOS increased concentration of iso-butyrate in comparison to the control birds, and it caused an increase in iso-valerate level, as compared to the probiotic group. A higher concentration of total SCFA

was noted in the group fed diet with probiotic preparation (P<0.05 *versus* control treatment). The experimental feeding with diets containing MOS or both supplements caused only a numerical increase in total SCFA concentration. In the group fed a diet containing both probiotic and MOS a higher concentration of propionate (significant differences as compared to other groups) and lower concentration of butyrate (significant difference with the control group) were determined.

Table 3. **Activity of microbial enzymes in the caecal digesta, µmol/h/g**

	Experimental groups				PooledSEM	*P*
	Control	Probiotic	MOS	Probiotic+MOS		
α-glucosidase	43.2^{b}	44.5^{b}	52.7^{b}	82.3^{a}	3.940	0.000
β-glucosidase	22.5^{a}	18.7^{ab}	17.7^{b}	19.5^{ab}	0.760	0.044
α-galactosidase	68.1^{b}	81.3^{b}	70.5^{b}	140^{a}	8.442	0.001
β-galactosidase	135^{b}	137^{b}	138^{b}	220^{a}	10.65	0.002
β-glucuronidase	40.7^{a}	35.9^{ab}	23.7^{c}	25.5^{bc}	2.277	0.009

Values with the different letters differ significantly; abc – $P \leq 0.05$

Table 4. **Short chain fatty acids concentration (µmol/g) and profile (%) in the caecal content**

	Experimental groups				PooledSEM	*P*
	Control	Probiotic	MOS	Probiotic+MOS		
Concentration						
acetate	46.4^{b}	57.3^{a}	52.6^{ab}	52.7^{ab}	1.500	0.048
propionate	19.2^{b}	23.6^{ab}	21.2^{bc}	25.1^{a}	0.722	0.007
iso-butyrate	0.92^{b}	0.95^{b}	1.11^{ab}	1.23^{a}	0.049	0.035
butyrate	13.1	13.5	12.4	10.7	0.557	0.309
isovalerate	1.31^{ab}	1.19^{b}	1.54^{ab}	1.64^{a}	0.074	0.039
valerate	1.51	1.66	1.53	1.51	0.054	0.765
total	82.3^{b}	98.2^{a}	90.4^{ab}	92.8^{ab}	2.373	0.048
Profile						
acetate	56.2	58.4	58.1	56.8	0.450	0.267
propionate	23.6^{b}	24.0^{b}	23.4^{b}	27.1^{a}	0.576	0.044
butyrate	15.6^{a}	13.7^{ab}	13.9^{ab}	11.4^{b}	0.519	0.024

Values with the different letters differ significantly; abc – $P \leq 0.05$

Discussion. Probiotic has been defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller, 1989). It is assumed as an important dietary factor, mainly probiotic lactobacilli and bifidobacteria species that inhibits growth of pathogens in the gastrointestinal tract as a reaction to a low pH of digesta, caused by increased production of short chain fatty acids (Rolfe, 2000; Patterson and Burkholder, 2003). In the present experiment, application of probiotic preparation as single supplement in laying hens' diet had no significant effect on the glycolytic activity of caecal microflora. At the same time, it increased concentrations of acetate and total SCFA, which resulted in a numerically, although not statistically confirmed, lower caecal pH.

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). The results of some experiments indicate that mannanoligosaccharides are capable to increase the intestinal number of lactobacilli and bifidobacteria (Baurhoo et al., 2007). Authors of other experiments indicated that the inclusion of MOS did not show a clear positive effect on total anaerobic bacteria, lactic acid bacteria, and *Clostridium perfringens* (Yang et al., 2007), as well as that commonly used low-dose MOS in the diet does not affect the intestinal ecosystem functioning (Juskiewicz et al., 2006). In our earlier study, a beneficial decrease in β-glucuronidase (marker enzyme for pathogenic microflora) activity followed a higher, than that in the present study, dosage of MOS (Juskiewicz et al., 2003). In the present experiment, application of MOS in laying hens diet had no significant effect on the glycolytic activity of intestinal microflora, concentration of SCFA and pH of caecal digesta. A profitable effect application of MOS in the diets was the reduction of the activity of microbial β-glucosidase and β-glucuronidase. Beside the aforementioned effects of applied single treatments with probiotic or prebiotic, a dietary combination of probiotic *Pediococcus acidilactici* with prebiotic MOS caused an additional effect in the caeca of hens beneficially reducing ammonia (toxic at high level) concentration and β-glucuronidase activity as well as

increasing propionate (known as factor beneficially affecting lipid metabolism) concentration.

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