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Influence of a prebiotic feed additive on some biochemical indices of blood and intestinal microbiota of broiler chickens

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Investigations were carried out in 2005 at the Research Laboratory of Biological Diversity and Technologies of Vilnius Pedagogical University and on the poultry farm "Petkus". The object of the study was chicken broilers divided into two groups (control and trial) each containing 100 day-old chickens (50 male and 50 female). Chickens were fed dry standard feed of the same composition and nutritional value in both groups, but to the feed of the trial group the prebiotic feed additive BIO-MOS was added. The dosage of the prebiotic additive was different at various periods of age of the chickens: 0–7 d. (2 kg/t feed), 7–35 d. (1 kg/t feed) and at 35–39 d. (0.5 kg/t feed).

The blood of the chickens at the age of 21 and 39 days was examined by the colorimetric method for total proteins and total nucleic acids. Serum protein fractions were investigated by electroforesis. Microbiological investigations of the intestinal content were carried out by standard methods.

Results of our study showed that in the trial group the total amount of nucleoacids in the blood of male chickens increased by 26.34% ($P < 0.01$) at 21 days of age in comparison with the control group.

Total proteins in the serum of male and female chickens of the trial groups increased by 3% during all the trial period in comparison with the control group. The level of globulins in the serum of the trial group male chickens at the age of 21 and 39 days increased by 2.3% and 3.3% and in the serum of female chickens by 1.30% and 1% in comparison with the control group. Analysis of different fractions of serum globulins showed that in the serum of the trial group chickens the content of γ fraction globulins was by 2.42% higher in comparison with the control group. A positive effect on the population of „beneficial" bacteria in the intestinal tract of chickens from the trial group was stated after the microbiological analysis of their gut content.

Addition of the prebiotic BIO-MOS to the feed had a positive influence on some biochemical indices of blood which are important for a good immunity and growth of chicken broilers.

Key words: chicken broilers, prebiotic feed additive, blood serum, protein content, nucleic acids, globulins, gut microflora

INTRODUCTION

World Health and Agriculture Organization (FAO) is currently creating a global food safety programme "Codex Alimentarius" which also includes a list of feed ingredients posing a risk for human health [7]. Public concern about the food safety lead to a decreased use of antibiotic growth promoters (AGP) in feed and subsequently to an increasing interest in alternative safe feed additives similarly enhancing the productivity of animals and their resistance to diseases [2, 13].

The productivity of broilers was increased significantly via genetical improvement but, on the other hand, they became more susceptible to various pathogens, especially to enteropathic microbes such as *E. coli, Salmonella* spp., *Clostridium perfringens* and *Campylobacter* spp. Wide use of antimicrobial growth promoters in feed, applying therapeutic and subtherapeutic dosages, was introduced in attempt to control these infections. There is a delicate balance of beneficial and pathogenic bacteria in the gastrointestinal tract (GIT) influenced by many symbiotic and competitive interactions [2].

Several mechanisms are described by which the normal population of bacteria in the GIT protects the host and enhances productivity: production of certain substances such as volatile fatty acids which inhibit multiplication of non-indigenous microorganisms, competition with non-indigenous organisms for nutrients available in limited supply, and competition for available tissue attachment sites in the GIT [4].

Since the ban of some antibiotic growth promoters in Europe in 1999, the search of alternatives became an important topic of research. The following candidates for alternatives were chosen: probiotics, prebiotics (non-digestible oligosaccharides), organic acids, enzymes and fitogenic modifiers of microbial activity [11].

Some of these substances have already been studied extensively in humans and others in animals [5, 9, 12]. In the past years, many studies were conducted to investigate the influence of probiotics, but prebiotics are the most recent subject of research.

Prebiotics are feed igredients not hydrolized and not resorbed in the small intestines and therefore reach the colon where they are fermented by a specific group of indigenous bacteria. Prebiotics were defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited group of bacteria in the colon, leading to an improvement in host health" [3]. So, prebiotics are used in order to provide a substrate for beneficial GIT bacteria (*Bifidobacterium* spp.*; Lactobacillus* spp.*,* etc.). Most of the substances investigated so far were forms of carbohydrates [8, 11].

Some bacteria in the large intestines are more specialized in hydrolizing plant polysaccharides and may produce small molecular weight polymers from large polymers. Certain non-starch polysaccharides are now recognized as having prebiotic activity in the large intestines [8, 11].

Sources of natural prebiotics are the seeds of leguminosae (soya, peas, broad beans, lupins), yeasts in whose walls mannano-oligosacharides (MOS) well known for their prebiotic activity are widely presented. Synthetic prebiotics are obtained from polymerization of the dysaccharides or from complex carbohydrates (fructo-oligosaccharides, galacto-oligosaccharides: FOS and GOS) via enzymatic hidrolysis [8].

Prebiotics in human diets have been shown to bring out "bifidogenic" effects and a shift in microbial metabolism from "proteolytic" to the more favourable "saccharolytic" [3]. This stimulation of saccharolytic activities leads to a reduced formation of toxic substances, such as ammonia, hydrogen sulfide, indoles and secondary bile acids. Another aspect of the potential usefulness of oligosaccharides as prebiotics is their immunomodulating effect. Indeed, mannose oligosaccharides have been shown to agglutinate pathogens and to function as an alternative to at least part of the AGP effect [10].

From studies in experimental animals, it has been shown that non-digestible oligosaccharides (NDO) and other fermentable carbohydrates can improve mineral absorption (e.g., calcium and magnesium) probably by increasing their solubility in the intestine following microbial fermentation.

A better knowledge of NDO will probably lead to applications for specific NDO. Trials performed in the USA and other countries have shown that MOS can improve growth and feed conversion ratio (FCR) in chickens. In order to exert their action on immunology, it should be noted that this NDO needs to act before fermentation and/or has to escape fermentation. It is stated that MOS is not fermented and can prevent bacterial infection via a mechanism which is quite different from that of antibiotics. Spring [10] indicated a 50% reduction in caecal salmonella contamination in chickens given MOS.

Many publications include data showing an important role of gut microflora in maintaining the immune homeostasis of the GIT and protecting it from inflammation. The composition of the feed greatly influences the characteristics of intestinal microflora [11].

Future investigations can prove that also other (mannan) carbohydrates have a similar effect. There is a lot of interest in the effect of these substances on the immune response [13].

The aim of our study was to investigate the effect of a novel prebiotic feed additive, mannano-oligosaccharide BIO-MOS, on some biochemical blood characteristics and gut microbiota influencing the resistance and performance of broiler chicken. This feed additive is produced from the external wall of yeast cells and contains phosphorilized glucomannans.

METHODS AND CONDITIONS

The investigations were carried out in 2005 at the Research Laboratory of Biological Diversity and Technologies of Vilnius Pedagogical University and on the poultry farm "Petkus". The object of the research were Ross chicken broilers.

Two groups were assessed, each of them containing 100 day-old chicken (50 male and 50 female). The first group was the control one and the second was the trial group.

Chicken were fed dry not granulated standard feed of the same composition and nutritional value in both groups, except addition of the prebiotic feed additive BIO-MOS for group 2. This additive was included into feed premixes for chickens from the first day of life to 39 days of age. The dosage of the prebiotic additive was different at various periods of the age of the chicken: 0–7 d. (2 kg/t feed), 7–35 d. (1 kg/t feed) and at 35–39 d. (0.5 kg/t feed).

The conditions of housing and management of chicken broilers were the same in both groups. Chickens were kept on deep litter and received water from stationary watering equipment from the first day of age till the age of 39 days.

The blood of chicken at the age of 21 and 39 days was examined for total proteins and for total nucleic acids, using the colorimetric method. Total proteins were assessed using a refractometer (IR-23), and for total nucleic acids the method of [14] was used. Fractions of serum proteins were investigated by the method of electroforesis, using the CORMAY GEL PROTEIN 100 protein plates [14]. They were scanned with a DVSE Sebia densitometer. Microbiological investigations of the intestinal content were carried out according to the standard methods LST 1432:1996 and LST ISO 7937:1998.

The data were processed by the standard method of statistical analysis. Statistical reliability of the data was estimated according to the t_x criterion (Version 1.8.1.ISBN 3-900051-00-3).

Scientific investigations were carried out in accordance with the Law of the Republic of Lithuania on animal care, housing and use [6].

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RESULTS AND DISCUSSION

Nucleoacid content in blood partly reflects the intensity of protein metabolism in the organism of chickens. The biological role of nucleoacids is participation in the synthesis of proteins; they are necessary in the synthesis of protein molecules. Results of our study showed that in the trial group, total nucleoacids in the blood of male chicken increased by 26.34% ($P < 0.01$) at 21 days of age in comparison with the control group (Table 1). In male chicken at 39 days of age as well as in female chicken during both periods of growth this result was close to that of the control group.

The increased of nucleoacid content is correlates with a higher level of proteins in the blood of chickens and shows that in the general the biosynthesis of proteins in the poultry organism has become more intensive. This is also confirmed by other blood biochemical parameters monitored during the trial.

Results of the study also showed that in the blood serum of chickens of the trial group the total amount of proteins increased. Total proteins in the blood of male and female chickens of the trial group at 21 days of age increased by 3.20% and 1.70% in comparison with the control group. The same tendency was observed at 39 days of age when total proteins in the blood of male and female chickens of the trial group were higher by 3.97% and 2.76% in comparison with the control group (Table 2). Changes in total proteins also reflect a more intensive metabolism of proteins in the chicken's organism.

Another important indicator of the physiological status is the proportion of different proteins in the blood. Blood proteins transport metabolism products, hormones, fatty acids, microelements and other substances [1]. Results of our study showed that in the trial group the content of albumin in the blood of male chickens increased by 2.3% at 21 days of age in comparison with the control group (Tables 3, 4). This result in male as well as female chickens at 39 days was respectively lower by 3.3% and 1% in comparison with the control group.

At the age of 21 d. and 39 d. the content of globulins in the blood serum of chickens of the trial group was higher in comparison with that in the control group (Tables 3, 4). At these periods of age, under the influence of the prebiotic additive BIO-MOS, globulin content in the blood serum of male chickens was higher respectively by 2.3% and 3.3% and in female chickens by 1.30% and 1.0% in comparison with the control group.

Three main fractions of globulins, α, β and γ, can be analysed in blood proteins by electrophoresis.

Table 1. **Total nucleic acids in the blood of chicken broilers, mg/%**

Group	Feeding characteristics	Age of 21 days		Age of 39 days	
		Male	Female	Male	Female
1	Standard feed – control	932.42 ± 162.902	1254.46 ± 54.463	1125.16 ± 104.053	1139.00 ± 17.624
2	Standard feed + BIO-MOS	1178.04* ± 101.554	1179.93 ± 164.110	1087.46 ± 215.549	1124.00 ± 85.465

Note. The difference between the control group and a corresponding group in the experiment is statistically reliable: *$P < 0.01$.

Table 2. **Total proteins in the blood serum of chicken broilers, g/l**

Group	Feeding characteristics	Age of 21 days		Age of 39 days	
		Male	Female	Male	Female
1	Standard feed – control	32.30 ± 1.612	35.73 ± 0.601	32.70 ± 2.080	31.37 ± 0.486
2	Standard feed + BIO-MOS	33.33 ± 0.513	32.77 ± 0.993	34.00 ± 1.702	32.23 ± 0.858

Table 3. **Protein fractions in the serum of chicken broilers at the age of 21 days, %**

Group	Feeding characteristics	Albumins		Globulins		Globulin fractions									
						Male					Female				
		Male	Female	Male	Female	α_1	α_2	β_1	β_2	γ	α_1	α_2	β_1	β_2	γ
1	Standard feed – control	48.30	47.80	49.40	52.20	2.4	23.9	2.9	4.6	17.0	2.3	22.3	5.0	3.2	19.4
2	Standard feed + BIO-MOS	50.60	46.50	51.70	53.50	3.1	22.7	3.2	3.7	17.6	1.7	22.2	1.9	3.3	24.4

Table 4. **Protein fractions in the serum of chicken broilers at the age of 39 days, %**

Group	Feeding characteristics	Albumins		Globulins		Globulin fractions									
						Male					Female				
		Male	Female	Male	Female	α_1	α_2	β_1	β_2	γ	α_1	α_2	β_1	β_2	γ
1	Standard feed – control	49.1	46.20	50.90	53.80	0.6	23.5	3.0	5.8	18.0	0.1	20.7	5.0	13.8	14.2
2	Standard feed + BIO-MOS	45.80	45.20	54.20	54.80	1.9	23.2	4.3	4.2	20.6	1.4	25.3	2.3	10.1	15.7

Table 5. **Results of microbiological investigation of chicken broiler gut contents**

No.	Microflora	Male		Female	
		Feeding characteristics			
		Standard feed – control group	Standard feed + BIO-MOS	Standard feed – control group	Standard feed + BIO-MOS
1.	Pathogenic enterobacteria	negative	negative	negative	negative
2.	Total *E. coli* counts	7.5×10^7	9.4×10^7	3.3×10^7	8.3×10^7
3.	Non-decomposing lactosis *E. coli* counts	8.1×10^7	8.0×10^7	5.1×10^7	6.4×10^7
4.	Hemolytic *E. coli* counts	negative	negative	negative	negative
5.	*Proteus* spp. group	10^3	10^3	10^3	10^3
6.	Other relatively pathogenic enterobacteria (*Citrobacter freundii, Citrobacter diversus*)	negative	negative	negative	negative
7.	Staphylococci (*S. sapropfyticus, S. epidermidis*)	9.0×10^4	negative	negative	negative
8.	*Staphylococcus aureus*	$<10^3$	$<10^3$	$<10^3$	$<10^3$
9.	Funghi	$<10^3$	$<10^3$	$<10^3$	$<10^3$
10.	Enterococci	3.5×10^5	5.2×10^5	7.6×10^5	3.0×10^6
		Found in dilution			
11.	Bifidobacteria (*Bifidobacterium* spp.)	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$
12.	Lactobacteria (*Lactobacillus* spp.)	10^{-7}	10^{-9}	10^{-7}	10^{-9}
13.	Bacteroids	10^{-9}	10^{-7}	10^{-9}	10^{-7}
14.	Clostridia (*Clostridium* spp.)	negative	negative	negative	negative

Globulins of α and β fractions are carriers of various substances, participate in the reactions of blood clotting, γ fraction globulins participate in the immune reactions and are particularly important for natural resistence of poultry [1]. Results of our trial showed that proportions of different globulin fractions can vary greatly.

The prebiotic feed additive had the biggest influence on the γ fraction of globulins in the serum. The content of γ globulins in the serum of the trial group male chickens at 21 and 39 days of age increased by 0.6% and 2.6% and in the serum of female chicken by 5.0% and 1.5%, respectively, in comparison with the control group results (Tables 3, 4).

In general, results of our trial imply that components of the prebiotic feed additive BIO-MOS help to maintain the microflora balance of the intestinal tract of poultry, resulting in a more efficient use of nutrients from the feed, more intensive processes of protein metabolism and subsequently in better health (Table 5).

As regards enteric microflora, samples of both groups were negative for pathogenic enteric bacteria (Table 5). The prebiotic feed additive BIO-MOS used in our investigation had no negative influence on bifidobacteria population in the intestinal tract of chicken of the trial group. Also, the numbers of "beneficial" lactobacteria in the GIT of chickens of the trial group increased by two steps in the dilution in comparison with the control group. However, the numbers of "non-beneficial" *Bacteroides* spp. were higher in the GIT of chickens from the control group (Table 5). These results clearly indicate a selective positive effect of the prebiotic BIO-MOS on the beneficial GIT microflora.

Results of our investigation in general indicate that there are good future possibilities for alternative growth-promoting feed additives such as BIO-MOS in successfully replacing the antimicrobial growth promoters. Future cientific investigations can also develop other prebiotic and phytogenic feed additives allowing to compose antibiotic-free feed ratios and keeping the same level of productivity.

CONCLUSIONS

1. Results of our study showed that in the trial group which received feed with addition of the prebiotic BIO-MOS, total nucleoacids in the blood of male chickens increased by 26.34% ($P < 0.01$) at 21 days of age in comparison with the control group. In male chickens at 39 days of age as well as in female chickens during both periods of growth, this result was close to that of the control group.

2. Total proteins in the serum of male and female chickens of the trial groups increased by 3% during all the trial period in comparison with the control group.

3. Serum globulins of male chickens in the trial group at the age of 21 and 39 days increased by 2.3% and 3.3% and of female chickens by 1.30% and 1% respectively in comparison with the control group.

4. Investigation of different fractions of serum globulins showed that chickens of the trial group had significantly higher levels (by 2,42%) of γ fraction globulins at the age of 21 and 39 days in comparison with the control group.

5. Addition of the prebiotic feed additive BIO-MOS to the feed had a positive effect on the population of "beneficial" bacteria in the intestinal tract of chickens from the trial group.

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PREBIOTINIO PREPARATO ĮTAKA VIŠČIUKŲ BROILERIŲ KRAUJO BIOCHEMINIAMS RODIKLIAMS IR ŽARNYNO MIKROFLORAI

S a n t r a u k a

Tyrimai atlikti 2005 m. Vilniaus pedagoginio universiteto Biologinės įvairovės ir technologijų laboratorijoje ir įmonėje „Petkus". Bandyme buvo sudarytos 2 grupės po 100 vienadienių viščiukų (50 gaidžiukų ir 50 vištaičių). Pirma grupė buvo kontrolinė, o kita – bandomoji. Kontrolinės grupės viščiukai broileriai buvo lesinami standartiniais kombinuotaisiais lesalais. Bandomosios grupės viščiukai buvo lesinami tokios pat sudėties ir maistingumo lesalais kaip ir kontrolinės, tik juose buvo įmaišyta prebiotinio preparato BIO-MOS. Šio preparato dozė įvairiais

viščiukų amžiaus periodais buvo skirtinga: 0–7 d. (2 kg/t lesalų), 7–35 d. (1 kg/t lesalų) ir 35–39 d. (0,5 kg/t lesalų).

Bandymo eigoje tirti 21 ir 39 dienų viščiukų broilerių kraujo biocheminiai rodikliai: bendras nukleorūgščių ir bendrųjų baltymų kiekis – kolorimetriniu metodu, baltymų frakcijos – elektroforezės būdu. Žarnų turinio mikrobiologiniai tyrimai atlikti pagal galiojančias standartines metodikas.

Nustatyta, kad bendras nukleorūgščių kiekis 21 dienos gaidžiukų kraujo serume padidėjo 26,34% ($P < 0,01$), palyginus su kontroline grupe. Dėl prebiotinio preparato įtakos per visą auginimo periodą vidutiniškai 3% padidėjo bendrųjų baltymų kiekis viščiukų broilerių kraujo serume. 21 ir 39 dienų gaidžiukų kraujo serume globulinų kiekis padidėjo atitinkamai 2,3 ir 3,3%, o vištaičių – 1,30 ir 1%, γ globulinų kiekis – vidutiniškai 2,42%, palyginus su kontroline grupe.

Tirtas prebiotinis preparatas BIO-MOS teigiamai paveikė kai kuriuos viščiukų broilerių kraujo biocheminius rodiklius, kurie svarbūs natūraliam organizmo rezistentiškumui ir viščiukų broilerių augimui.

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Raktažodžiai: viščiukai broileriai, prebiotikai, kraujo serumas, bendrieji baltymai, nukleorūgštys, globulinai, žarnyno mikroflora

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ВЛИЯНИЕ ПРЕБИОТИКА НА БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ КРОВИ И МИКРОФЛОРУ КИШЕЧНИКА У ЦЫПЛЯТ-БРОЙЛЕРОВ

Р е з ю м е

Исследования проведены в 2005 г. в Лаборатории биологической разновидности и технологий Вильнюсского педагогического университета и на предприятии „Petkus“. В опыте были скомплектованы 2 группы: в каждой группе по 100 1-дневных цыплят-бройлеров. Первая группа – контрольная, а вторая – опытная. Цыплята контрольной группы получали стандартный корм, а цыплята опытной – корм такого же состава и питательности, что и цыплята контрольной группы, но в состав корма вводился пребиотик BIO-MOS. Для каждого возрастного периода дозы пребиотика были разные.

Результаты исследований показали, что у 21-дневных петушков опытной группы общее количество нуклеокислот в сыворотке крови на 26,34% ($P > 0,01$) выше, чем в контрольной группе. Пребиотик BIO-MOS положительно влиял на общее количество белков в сыворотке крови у цыплят-бройлеров. В опытной группе глобулин у 21- и 39-дневных петушков был выше на 2,3 и 3,3%, а у кур соответственно на 1,30 и 1,00%, чем в контрольной группе.

На основании полученных нами результатов можно сделать вывод о том, что пребиотик BIO-MOS положительно влиял на биохимические показатели крови, которые важны для натуральной сопротивляемости роста цыплят-бройлеров.

Исследования финансировались Литовским государственным фондом науки и образования.

Ключевые слова: цыплята-бройлеры, пребиотики, сыворотка крови, общее количество белков, нуклеокислоты, глобулины, микрофлора кишечника