

**STUDY THE EFFECT OF USING PROBIOTIC  
(VETLACTOFLOSUM) ON SOME OF BIOCHEMICAL AND  
IMMUNOLOGICAL PARAMETERS OF BROILER CHICKENS**

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**ABSTRACT**

In the present study its used liquid preparation of probiotics "Vetlactoflorum-M" (dissolved in diluted milk) and "Vetlactoflorum-C" (dissolved in whey), and were studied its effect on some parameters such as total protein, globulin, albumin also serum bactericidal and lysozyme activity.

One hundred fifty broilers chicken breed "Ross-308" used in one day old. They were divided into three groups of 50 chicks (control "un-supplemented with probiotic ", first treatment group received "Vetlactoflorum-C" and second treatment group received "Vetlactoflorum-M"). Blood samples were taken in 7, 14, 21, 28, 35 and 42 day-old. Laboratorial analyses were conducted in Vitebsk state academy of veterinary medicine in Belarus. Level of total protein in the 2<sup>nd</sup> group to the end of the study was higher than in control group to 8.1% (P <0.05) in the third group by 3.96% (P <0.01). During observation period in chicken's serum of supplemented probiotic groups, albumin concentration was higher relative to the control group. Similar results were observed in level of globulins. In day 42 of broilers chicken, globulin content in both treatment groups compared with the control group as higher by 2.97% (P<0.01) and 5.19% (P <0.05), respectively. The level of serum bactericidal activity in 42 day of 2<sup>nd</sup> group was higher than the control at 23.62% (P <0.01), and in the third group by 16.21% (P> 0, 05). The index of serum lysozymal activity also surpassed control group in chickens in group 2 by 11.96% (P> 0.05), and in the third group at 10.69% (P> 0.05).

## INTRODUCTION

Pasteur suggested that microorganisms are necessary for normal life (1), it has only been in the past several decades that the microflora of the gastrointestinal tract has generated much interest amongst researchers. With the exception of pathogens and the diseases that they cause, there was little appreciation of the normal intestinal microflora. (2).

Since 1<sup>st</sup> January 2006 onwards, the European Union (EU) has decided to ban antibiotics as feed additives (3). Hence, probiotics have been considered for alternative to antibiotic. Probiotics are microorganisms that are fed to animals to colonize the intestinal environment and promote a better flora balance (4). Besides, these microorganisms are responsible for production of vitamins B complex and digestive enzymes, and for stimulation of intestinal mucosa immunity, increasing protection against toxins produced by pathogenic microorganisms.

Probiotics consisting of live or dead organisms and spores (5), non-traditional chemicals (6), bacteriophages (7) and others have emerged in the last decades as some of the tools that could be potentially useful in the near future for pathogen control and poultry performance improvement.

(8) has reported that probiotics stimulate the immunity of the chickens in two ways (a) flora from probiotic migrate throughout the gut wall and multiply to a limited extent or (b) antigen released by the dead organisms are absorbed and thus stimulate the immune system. At present it is believed that there is some relationship between the ability of strain to translocate and the ability to be immunogenic. The improvement in the immune system may be by three different ways: (a) enhanced macrophage activity and disturbance and enhanced ability to phagocytosis microorganism or carbon particles; (b) increased production of antibodies usually of IgG & IgM classes and interferon (a nonspecific antiviral agent) and; (c) increased local antibodies at mucosal surfaces such as the gut wall (usually IgA) (9).

Limited liability company "Microbiotic" Vitebsk region made probiotic preparation "Vetlactoflorum". However, given the preparation has not been studied previously in the broiler chickens. We were the first in Belarus began the study of the preparation, which was approved by the result of scientific and technical papers (10, 11, 12, 13).

Vetlactoflorum-liquid preparation of live probiotic acidophilus bacteria strain *Lactobacillus acidophilus* EP 317/402 "Narine" containing 1 cm<sup>3</sup> of not less than 10<sup>7</sup> colony forming units of Lactobacillus.

The aim of this study was to extend our research and examine the effect of a defined probiotic on blood protein concentration of broilers chicken and serum bactericidal and lysozyme activity.

## **MATERIALS AND METHODS**

Experimental study was conducted by our clinic Epizootology Department, Department of Microbiology and Virology, Veterinary and Sanitary Inspection and laboratory in Scientific Research Institute of Veterinary Medicine and biotechnology/Vitebsk State Academy of Veterinary Medicine.

For laboratory study, 150 broiler chicks breed "Ross-308" were used, this chicks divided into three groups 50 broilers chickens in each, acquired by "Vitebsk broiler chickens farm". The broiler chickens were reared under same environmental conditions, they were kept in thermo-neutral hall (approximately from day one old 33°C until final 19°C). In closed hall thermo aggregate was installed and experimental conditions with defined temperature and humidity were monitored by thermostat.

Chicks one day-old are sorted by gender and experience for the composition of the principle of analogy on 25 chicks' males and females in each group. The feeding period lasted in 42 days. Feed and water were provided *ad libitum*.

Administration of treatments: Throughout the experiment, broiler chickens given probiotic with drinking water according follow:

<b>№ group</b>	<b>Diet and treatment</b>
<b>Group 1 (control)</b>	Basic Diet.
<b>Group 2</b>	Basic Diet + probiotic "Vetlactoflorum-M" daily drinking water at a dose of 0.1 ml / bird (1-27day) and 0.2 ml / bird (28-42 days).
<b>Group 3</b>	Basic Diet + probiotic "Vetlactoflorum-C" daily drinking water at a dose of 0.1 ml / bird (1-27day) and 0.2 ml / bird (28-42 days).

Collection of samples, data collection and analysis. At 7, 14, 21, 28, 35 and 42 days of age, 4 ml of blood for laboratory analysis were obtained by axillary vein (with aseptic and antiseptic) and decapitation, in two different sterile tubes (14, 15). In one of them stabilize blood anticoagulant Trilon B (disodium EDTA) of 0.1 - 0.2 ml of 10% solution in 10 ml blood, the other was without anticoagulant to obtain serum.

Serum obtained after coagulation at a temperature of (18-20)°C, then put tubes in centrifuge at 1500 RPM/min for 10-15 minutes to separate the serum. The serum was separated, and then stored at -20°C until assayed to measuring blood proteins, albumin, globulin, serum bacteriocidal and lysozymal activity.

The total serum protein levels were measured by biuret method by Eurolyser automatic biochemical analyzer using reagents recruitment firm Cormay (Poland), the protein content expressed as G/L. Determination of the concentration of albumin in the blood serum was standardized by the reaction with bromocresol green. Albumin and globulins are expressed in g/l.

Study these indicators were performed on serum biochemical analyzer brand Eurolyser using reagents recruitment firm Cormay (Poland) (16, 17).

Serum bactericidal activity (Basque) investigated by photonephelometric method on Munsel Treffensu and modified by Smirnova and Kuzmina (18). The results of this study were statistically analyzed to find the significant differences among the treated groups by Excel programme.

## RESULTS AND DISCUSSION

In the present study, results of blood analysis of broiler chickens show that the preparation, dissolved in milk and whey, throughout period of rearing has a positive effect on biochemical parameters of blood serum. Birds monitored throughout experimental period, which was being healthy. However, the most pronounced changes were observed in the group which given probiotic diluted in whey. Table 1 shows the content of the reliable indicators of serum total protein during the entire experiment. The level of total protein in the 2nd group to the end of the study was higher than in the blood of control group to 8.1% ( $P < 0.05$ ) in the third group - by 3.96% ( $P < 0.01$ ). During observation period in chicken's serum of supplemented probiotic groups, albumin concentration was higher relative to the control group. A similar trend was observed in determining the amount of globulins. In day 42 of life broilers globulin content in both the treatment groups compared with the control group as higher by 2.97% ( $P < 0.01$ ) and 5.19% ( $P < 0.05$ ), respectively. These results agree with (19) who reported that hyperproteinemia was due to hyperglobinemia as a result of using probiotic (*Pediococcus acidilactic*). However these results disagree with (20) who showed no significant differences in total protein, albumin and globulin between treatments with probiotics. Also results disagree with the results of (21) who reported no significant difference in the level of total proteins in a study with ducklings using a multi-strains probiotic.

The administration of probiotic bacteria in chickens was shown to enhance specific, systemic antibody response and to stimulate the production of natural antibodies such as serum IgG and IgM (22). Increase in globulin enhance immunity in experimental groups supplemented probiotic that agreed with (23) reported that birds treated with *L. reuteri* exhibited longer ileal villi and deeper crypts, which are a response, associated with enhanced T cell function and increased production of anti-Salmonella IgM antibodies. Also (24) found that *Lactobacillus* supplementation of layers diets increased cellularity of Peyer's patches in the ileum indicating a stimulation of the mucosal immune system that responded to antigenic stimuli by secreting immunoglobulin (IgA).

**Table (1) Biochemical parameters of blood serum for broiler chickens.**

AGE (DAY)	Group	Total Protein (g/l)	Albumin (g/l)	Globulin (g/l)	Albumin/Globulin (%)
7	Group 1 Control	21,86±0,476	16,32±1,474	5,68±1,6165	2,87±1,023
	Group 2	24,19±1,944	17,51±1,419** *	6,68±0,870	2,62±1,065
	Group 3	23,05±1,586	16,50±1,761	6,54±0,802	2,52±1,298
14	Group 1 Control	39,17±3,093	22,59±3,243	16,57±4,543	2,49±1,282
	Group 2	39,25±0,990	24,67±0,761	14,58±1,589	1,80±0,251
	Group 3	37,07±2,167	22,66±1,027	14,40±2,420	1,83±0,407
21	Group 1 Control	38,88±3,939	17,85±1,393	20,01±2,851	0,89±0,093
	Group 2	32,41±3,571	15,52±0,884	18,89±2,297	0,88±0,122
	Group 3	39,16±1,865	16,09±1,452	23,07±1,396	0,71±0,080
28	Group 1 Control	24,51±5,759	5,19±0,599	19,31±5,400	0,92±0,677
	Group 2	34,86±2,157	6,24±0,423	28,62±1,916	0,22±0,015
	Group 3	31,88±2,316	6,28±0,717*	24,62±2,949	0,26±0,092
35	Group 1 Control	31,26±3,257	5,45±0,910	25,81±2,729	0,36±0,091
	Group 2	36,61±3,413	9,40±1,154**	27,21±3,040	0,49±3,048
	Group 3	35,76±1,963	7,83±0,391*	27,92±1,918	0,49±0,062
42	Group 1 Control	32,77±1,102	9,41±1,880	23,36±2,050	0,40±0,091
	Group 2	35,66±0,924*	11,65±0,696	24,08±1,140**	0,49±0,048*
	Group 3	34,12±1,687**	9,48±0,829	24,64±1,575*	0,38±0,0619

Means of mark

\*, \*\*, \*\*\* means significant different compare with control (P<0.05), (P<0.01), (P<0.001) respectively.

2<sup>nd</sup> Group–diluted in milk.

3<sup>rd</sup> Group–diluted in whey.

The published data about use of probiotic which improve resistance of animals and birds showed that an important feature of probiotic is their ability to increase the body's resistance, have in some cases, anti-allergic effect, regulate and promote the factors of nonspecific resistance. (25) Reports that the use of probiotics and bacteria in broiler increases the local defense of the digestive tract, stimulates growth and reduces the use of antimicrobials.

The lactobacillus sp. and bacillus sp. enhance resistance to pathogens by activating both cellular and humeral defenses (26). Natural resistance mechanisms are formed and appear under the influence of a variety of external factors, some of which are components of the feed. Although the level of natural resistance can indirectly judge our studies on such indicators as blood cells, hemoglobin, total protein concentration, for a more objective characteristics determine the bactericidal activity of lysozyme and blood. Our studies have shown that Vetlactoflorum positively affect the level of bactericidal and lysozyme activity levels (Table 2). Thus, under the influence of Vetlactoflorum serum bactericidal activity increased. The intensity of these changes depends on the scheme of the preparation. The maximum effect is achieved with the use of the Vetlactoflorum-M at a dose of 0.1 ml / bird from 1day old to 27 day and 0.2 ml / bird 28 day old until the end of rearing. More pronounced changes serum bactericidal activity noted in the first 14, 28, 42 days.

In the experiment, there have been studies describing impact of probiotic on the performance of non-specific resistance of the body of birds (serum bactericidal and lysozymal activity). The study of these factors showed that preventive measures contributed to their increase. The level of serum bactericidal activity to 42 day of life in the 2<sup>nd</sup> experimental group was higher than the control value at 23.62% ( $P < 0.01$ ), and in the third group - by 16.21% ( $P > 0, 05$ ). Serum lysozyme activity of broiler chickens in experimental groups throughout the experiment, with varying degrees of reliability was higher than that of the control group of birds (also table 2) in 2<sup>nd</sup> group by 11.96% ( $P > 0.05$ ), and in the third group at 10.69% ( $P > 0.05$ ).

Lysozyme plays an essential role in the regulation of cell differentiation and proliferation, in providing immune-structure tissue homeostasis. Lysozyme is an enzyme with antibacterial activity that can split peptidoglycanin bacterial cell walls particularly the gram species and it can cause lysis of the cells (27). However, it has a specific enzymatic action, and non-specific effects, is involved in the regulation of the permeability of tissue barriers. From the literature it is known that freshly serum has a different degree of bacteriostatic and bactericidal against many types of microorganisms. Bactericidal due to the presence in it bacteriolysin, complement, lysozyme, properdin, interferon, leukocytes. Nonspecific bacteriolysis is lysozyme, enhances bactericidal bacteriolysin. Thus, the bactericidal activity of blood and serum is a summary measure of non-specific humoral immunity. In the present study, the increase in lysozyme activity of broiler chickens suggests an immune stimulation. Infections or invasion by foreign material could results in an increase in the lysozyme concentration in fish blood (28, 29)

The activation mechanisms involved are known to be related to the polysaccharide from the cell wall. Similar finding were reviewed by Robertsen (30)

**Table (2) Natural resistance blood serum of broiler chickens.**

<b>AGE (DAY)</b>	<b>Group</b>	<b>Serum bacteriocidal activity</b>	<b>Serum Lysozymal activity</b>
7	<b>Group 1 Control</b>	11,37±1,196	18,55±0,674
	<b>Group 2</b>	12,092±1,145	21,85±0,791*
	<b>Group 3</b>	12,296±1,804	21,092±1,161
14	<b>Group 1 Control</b>	10,86±0,674	18,55±0,674
	<b>Group 2</b>	14,27±1,06**	21,85±0,791*
	<b>Group 3</b>	12,68±1,022	21,09±1,161
21	<b>Group 1 Control</b>	18,28±2,707	26,40±0,427
	<b>Group 2</b>	16,21±1,528	28,34±0,320
	<b>Group 3</b>	16,19±3,169	28,54±0,603
28	<b>Group 1 Control</b>	15,51±3,247	27,06±0,304
	<b>Group 2</b>	17,32±2,571**	27,62±0,190*
	<b>Group 3</b>	17,47±0,974*	26,5±0,363
35	<b>Group 1 Control</b>	16,32±0,875	25,17±0,162
	<b>Group 2</b>	18,59±0,624	26,54±0,37*
	<b>Group 3</b>	17,42±0,748	26,33±0,48
42	<b>Group 1 Control</b>	17,00±1,400	22,22±0,76
	<b>Group 2</b>	22,26±15,308*	25,24±0,446
	<b>Group 3</b>	20,29±2,873	24,88±0,466

Means of mark

\*, \*\*, \*\*\* means significant different compare with control (P<0.05), (P<0.01), (P<0.001) respectively.

2<sup>nd</sup> Group–diluted in milk.

3<sup>rd</sup> Group–diluted in whey.

## دراسة تأثير استخدام المعزز الحيوي (VETLACTOFLOSUM) على بعض المؤشرات الحيوية والمناعية في دجاج اللحم

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### الخلاصة

في الدراسة الحالية استخدم مستحضر سائل من "Vetlactoflorum-M" الذائب في الحليب، و "Vetlactoflorum-C" الذائب في شرش الحليب، و درست تأثيره على بعض المقاييس مثل البروتين الكلي والكلوبيولين والالبومين وكذلك النشاط القاتل للبكتريا والحال في مصل الدم.

أخذت ١٥٠ فروج لحم من سلالة "روس-٣٠٨" في عمر يوم واحد، وقسمت إلى ثلاث مجموعات كل مجموعة تتكون من ٥٠ فروجة لحم (مجموعة السيطرة لم تعطى المعزز الحيوي) ومجموعة المعالجة الاولى التي اعطيت المعزز الحيوي "Vetlactoflorum-C" ومجموعة المعالجة الثانية التي اعطيت المعزز الحيوي "Vetlactoflorum-M". تم أخذ عينات الدم في عمر ٧ و ١٤ و ٢١ و ٢٨ و ٣٥ و ٤٢ يوما. أجريت الدراسة في مختبرات اكااديمية فيتبسك الحكومية للطب البيطري في روسيا البيضاء. كان مستوى البروتين الكلي في المجموعة الثانية حتى نهاية الدراسة أعلى من مجموعة السيطرة بحوالي ٨.١% ( $P < 0.05$ ) في مجموعة التجربة الثالثة ٣.٩٦% ( $P < 0.01$ ). خلال فترة المراقبة لوحظ في مصل دجاج اللحم للمجموعتين اللتين اعطيت المعزز الحيوي ان تركيز الألبومين أعلى نسبة بالمقارنة مع مجموعة السيطرة. ولوحظت نتائج مماثلة في كمية الجلوبيولين. في عمر ٤٢ يوم كان مستوى الكلوبيولين في كلا مجموعتي المعالجة (الثانية والثالثة) بالمقارنة مع مجموعة السيطرة كان مرتفعا بنسبة ٢.٩٧% ( $P < 0.01$ ) و ٥.١٩% ( $P < 0.05$ ) على التوالي. وكان مستوى نشاط القاتل للجراثيم في مصل الدم في ٤٢ يوم من مجموعة التجربة كان أعلى من مجموعة السيطرة بحوالي ٢٣.٦٢% ( $P < 0.01$ )، وفي المجموعة الثالثة بحوالي ١٦.٢١% ( $P < 0.05$ ). تجاوزت مؤشر النشاط الحال في المصل أيضا مجموعة السيطرة في افراخ الدجاج في المجموعة الثانية من ١١.٩٦% ( $P > 0.05$ )، وفي المجموعة الثالثة في ١٠.٦٩% ( $P > 0.05$ ).

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