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## The influence of brovitatoxide in conjunction with milk thistle fruits on the immune system of turkeys for eimeriozic invasion

I. Khariv, B. Gutyj, V. Hunchak, N. Slobodyuk, A. Vynyarska, A. Sobolta, V. Todoriuk, R. Seniv  
chariv\_ii@ukr.net

*Lviv national university of veterinary medicine and biotechnologies named after S.Z. Gzhytskyj,  
Pekarska Str., 50, Lviv, 79010, Ukraine*

*The immune system provides resistance of the organism against bacterial and viral infections in the body of the poultry. In the intestinal mucosa of eimeria it was secrete metabolic products, that are toxic to various systems and tissues of turkeys. Eimeria, parasitizing in the gut, inhibit specific phase of immunity presented by antibodies (humoral type), reduce the activity of sensitized cells (cell type), slow down nonspecific phase of immunity, represented by various immune cells. The rapid and complete recovery of functional state of the immune system in turkeys, affected by eimeriozic invasion it was found if brovitatoxide was given if the aggregate of the fruits of milk thistle.*

*Fruits contain group of flavius lignans named «Sylimaryn», acting immune stimulatory for the development of secondary immuno deficiencies state of body.*

**Key words:** brovitatoxide, milk thistle, eimeria, immune system.

## Вплив бровітакоксиду сукупно з плодами розторопші плямистої на стан імунної системи індиків за еймеріозної інвазії

І.І. Харів, Б.В. Гутий, В.М. Гунчак, Н.М. Слободюк, А.В. Винярьська,  
А.Г. Соболта, В.Б. Тодорюк, Р.В. Сенів  
chariv\_ii@ukr.net

*Львівський національний університет ветеринарної медицини біотехнологій імені С.З. Гжицького,  
бул. Пекарська, 50, м. Львів, 79010, Україна*

*Імунна система забезпечує резистентність організму проти бактеріальних і вірусних інфекцій у організмі птиці. У слизовій оболонці кишечника еймерії виділяють продукти метаболізму, що діють токсично на різні системи і тканини індиків. Еймерії, паразитуючи в кишечнику, пригнічують специфічну фазу імунітету представлену антитілами (гуморальний тип), знижують активність сенсibilізованих клітин (клітинний тип), сповільнюють неспецифічну фазу імунітету, що представлена різними імунними клітинами. За виникнення еймеріозної інвазії, при застосуванні для лікування бровітакоксиду, на період клінічного одужання, стан клітинного і гуморального імунітетів суттєво поліпшився. Проте, на 5-у добу після одужання на 20% нижче нормальних величин була загальна кількість лімфоцитів та на 10% кількість Т- і В- лімфоцитів, що вказує на неповне відновлення функціонального стану клітинної ланки імунітету. Низька лізоцимна активність сироватки крові, та високий рівень у ній циркулюючих імунних комплексів на 5-у добу після клінічного одужання вказує на неповне відновлення функціонального стану гуморальної ланки імунітету. Підтвердженням цього є низька фагоцитарна активність нейтрофілів.*

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*Швидке і повне відновлення функціонального стану імунної системи у індиків, уражених еймеріозною інвазією встановлено якщо бровітакоксид додавали сукупно із плодами розторопші плямистої. Плоди містять групу флавонолігнанів під назвою «Силімарин», що діє імуностимулювально за розвитку вторинного імунодефіцитного стану організму.*

**Ключові слова:** бровітакоксид, розторопша плямиста, еймерія, імунна система

## Introduction

Ukraine has recently slightly reduced the number of breeding and commodity farms of the turkeys growing. However, livestock of aforementioned poultry was significantly increased in adjoining the farm economy (Kobcova, 2001). A significant increase in the volume of poultry production can be achieved under the conditions of use of evidence-based system of doing the industry. Of particular importance is the question of a comprehensive study of infectious and invasive diseases considering exactly edge Epizootology. The importance of this issue in terms of efficiency of early diagnosis and the development of effective therapy is emphasized in a number of scientific papers (Mashke and Zakharov, 2002; Bohach and Taranenko, 2003; Timofeev, 2004; Khariv, 2011).

In early phylogenetic development for turkey poults there are different stress factors – inadequate feeding, inadequate housing conditions, bacterial infections, worms and protozoon invasion. This leads to decrease in body's natural resistance and inhibition of bone marrow hematopoietic function (Kotel'nikov, 1991; Dakhno et al., 2001). To improve the immune status of animals and poultry in practice of veterinary medicine different immune stimulatory drugs were used, namely KAFI, T-activin, leucogen, homotine, immunoglobulins, thymogen, kamizol etc. (Mashke and Zakharov, 2002; Khariv, 2011; Gutyj, 2013; Guberuk et al., 2015; Hariv and Gutyj, 2016; Khariv et al., 2016; Martyshuk et al., 2016; Nazaruk et al., 2016; Gutyj et al., 2016; Gutyj et al., 2017). The disadvantage of these drugs is that they are introduced parenterally, as is well known, poultry up to 3 months of age hardly carry over parenteral injection.

To improve the immune status of the organism of turkeys, secure and easy to use are vegetable preparations, that is added to feed. Their immune stimulatory action is not inferior to that of chemical drugs, and shows «softer» immune stimulating effect. To herbal preparations, showing a high immune stimulating effect should include Echinacea herb and fruit of milk thistle. At the moment such plants are widely studied and used in medical practice of humane medicine, however they do not pay due attention to the practice of veterinary medicine, particularly in poultry. According to foreign literature given issue has been little studied, but native in Ukraine in this direction was developed for the first time.

The purpose of our research was to study the effect of only brovitatoxide, and with the combined use with the fruits of milk thistle on normalization of the immune system of turkeys by spontaneous affection of eymeriozic invasion.

The criteria of therapeutic effectiveness of brovitatoxide was dismissal of the turkeys organism from eymeria. Taking into account that sick turkeys with suppressed immune system, along with the use of brovita coccidia, milk thistle fruits was added with food to intensify cellular, humoral and nonspecific immunities. Besides, com-

pared the therapeutic effectiveness of both methods of turkeys treating for the occurrence of eymeriozic invasion.

## Materials and methods

To investigate the influence of brovitatoxide and the fruits of milk thistle at normalization of morphological and biochemical parameters of turkeys immune system by spontaneous affection with eymeriozic invasion, three groups of research turkeys to 20 poultries in each group were formed.

Turkeys of the first group were treated with the help of brovitatoxide which was added at a dose of 2 g/kg of feed. The second group of turkeys were added brovitatoxide 2g/kg and milled powder of milk thistle fruits of 2 g/kg of feed. Drugs were added with wet forage for 5 days in a row. The control group had normal blood parameters of the third group of clinically-healthy turkeys - analogue with compatible brooders. In each group it was noticed with ink 20 turkeys from which under the wing vein it was taken and after 3 and 5 days of treatment, and after 5 days after recovery. The blood was determined by the number of leukocytes, lymphocytes, T lymphocytes, B lymphocytes, lysozyme activity of serum, bactericidal activity of serum, circulating immune complexes, seromucoid level, phagocytic activity of neutrophils, phagocytic index and phagocytic number.

## Results and discussion

The immune system of animals and poultry provides resistance of the body against bacterial and viral infections. At helmintozic and protozoonotic diseases functional immune system is suppressed and there comes a secondary immunodeficiency.

In patients of turkeys (Table 1), because of toxins action, allocating eymeria, before treatment leukocytosis was set. The number of leukocytes was  $5.98 \pm 0.56$  g/l against  $3.45 \pm 0.14$  g/l in clinically healthy poultry, that is 73.3% higher ( $P < 0.001$ ).

On the 3rd day of brovitatoxide treatment their number decreased by 2 times, and on the fifth day was 12.3% greater than normal. Over 5 days after clinical recovery the number of leukocytes was 9% greater than control, which indicates the presence of inflammation of the mucous membranes in areas where eymeria parasitize.

In turkeys which were treated with brovitatoxide were slowly normalized the indicators of cellular immunity. Before treatment, the total number of lymphocytes was  $62.2 \pm 2.15$  g/l against  $88.6 \pm 2.2$  g/l in clinically healthy, which is 42.4% less ( $P < 0.001$ ). On the 3rd day of treatment the number of lymphocytes has remained at a low level, slightly raised on the fifth day (period of clinical recovery) and on the 10th day (5th day after treatment) their number was 20% less than the control.

Table 1

**The indicators cellular immunity in turkeys, affected by eymeriozic invasion and treated with brovitatoxide**  
(M ± m; n = 20)

| The indicators<br>Control / Experiment | Day of research               |                               |                              |                             |
|--|-------------------------------|-------------------------------|------------------------------|-----------------------------|
|  | The first                     | The third                     | The Fifth                    | The Tenth                   |
| Leucocytes,<br>G/L                     | 3.45 ± 0.14<br>5.98 ± 0.56*** | 3.54 ± 0.24<br>4.86 ± 0.23*** | 3.42 ± 0.12<br>3.84 ± 0.16*  | 3.45 ± 0.14<br>3.76 ± 0.12* |
| Lymphocytes,<br>G/L                    | 88.6 ± 2.2<br>62.2 ± 3.1***   | 86.2 ± 2.1<br>64.5 ± 2.0***   | 88.2 ± 1.8<br>70.5 ± 2.1*    | 87.4 ± 1.5<br>72.6 ± 1.4*   |
| T lymphocytes,<br>(E-RUK) g/l          | 0.12 ± 0.02<br>0.09 ± 0.01*** | 0.12 ± 0.03<br>0.09 ± 0.02*** | 0.11 ± 0.05<br>0.10 ± 0.03*  | 0.11 ± 0.04<br>0.10 ± 0.05* |
| B-lymphocytes (EAC-<br>RUK) g/l        | 0.32 ± 0.04<br>0.26 ± 0.06**  | 0.33 ± 0.06<br>0.28 ± 0.04*   | 0.32 ± 0.06<br>0.280 ± 0.06* | 0.33 ± 0.04<br>0.30 ± 0.06* |

In this and the following table, the degree of probability as compared to the control group: \* – P < 0.05, \*\* – P < 0.02, \*\*\* – P < 0.001

A number of lymphocytes populations returned to normal quite slowly. In particular, for the period of clinical recovery the number of lymphocytes was by 14.3% and T lymphocytes by 10.2% less than control values. At the same level, both of lymphocytes population were and on the fifth day after clinical recovery (Table 1).

At research of the quantities of humoral immunity In turkeys affected with eymeriozic invasion (Table. 2) it was set the decrease of antimicrobial activity of blood

serum and increase of CIC by 33% seromucoid by 89%, indicating the suppression of humoral immunity.

When applying brovitatoxide for the treatment of turkeys, for the period of clinical recovery (5-day), the indicators of humoral immunity were restored to normal values, but were lower than in clinically healthy poultry – bactericidal activity of serum by 13.9%, lysozyme activity of serum – by 19.1%, and indicators circulating immune complexes were high by 26% and seromucoid by 23.5% (Table. 2).

Table 2

**Indices of humoral immunity in turkey, affected with eymeriozic invasion and treated with brovitatoxide**  
(M ± m; n = 20)

| The indicators<br>Control / Experiment | Day of research               |                               |                              |                            |
|--|-------------------------------|-------------------------------|------------------------------|----------------------------|
|  | The first                     | The third                     | The Fifth                    | The Tenth                  |
| Lysozyme activity of<br>serum, %       | 26.4 ± 1.2<br>21.2 ± 1.4**    | 26.8 ± 1.4<br>21.4 ± 1.6**    | 26.8 ± 1.2<br>22.5 ± 1.4*    | 26.2 ± 1.4<br>23.6 ± 1.2*  |
| Bactericidal activity of<br>serum, %   | 68.5 ± 2.4<br>56.3 ± 2.6**    | 68.2 ± 1.8<br>58.5 ± 1.7*     | 68.8 ± 2.6<br>60.4 ± 1.4*    | 68.8 ± 2.4<br>64.6 ± 1.8   |
| Circulating immune<br>complexes, %     | 25.7 ± 2.3<br>34.3 ± 1.6***   | 25.4 ± 1.4<br>32.5 ± 1.2***   | 26.2 ± 1.2<br>31.7 ± 1.4**   | 25.8 ± 1.4<br>28.6 ± 1.2*  |
| Seromucoid mg/cm <sup>3</sup>          | 0.18 ± 0.03<br>0.34 ± 0.05*** | 0.18 ± 0.04<br>0.26 ± 0.06*** | 0.17 ± 0.05<br>0.21 ± 0.04** | 0.17 ± 0.04<br>0.18 ± 0.06 |

Lysozyme activity of blood serum in sick turkeys was by 24.5% lower than in clinically healthy poultry. At lower level it remained and on the 3<sup>rd</sup> –day, and slightly raised on the fifth day of treatment. But, and 5 days after clinical recovery lysozyme activity of serum was by 11% lower than the control.

Bactericidal activity of blood serum In turkeys before treatment was 21.6% lower than normal. It has increased by 3rd and 5th day of treatment, but, for the period of clinical recovery was 13.7% lower than the control, and was normalized only 5 days after clinical recovery.

The level of circulating immune complexes in blood in serum of sick turkeys was 33.4% higher compared to the clinically healthy poultry. It decreased slightly on the 3rd and 5th day of treatment, but was respectively 28% and 21% higher than the control. On the 10th day of the experiment CIC levels in serum remained 11% higher than in clinically healthy poultry.

Seromucoid level in serum of sick turkeys amounted to 0,34 ± 0,05 mg/cm<sup>3</sup>, that is 88.7% less than in clinically healthy – 0,18±0,03 mg/cm<sup>3</sup> (P < 0,001). On the 3rd day of treatment, seromucoid level in blood serum was decreased in 2 times, compared with the level before treatment. More significant reduced the level of seromucoid

on the fifth day of treatment, but was only by 23.5% higher than the control. Within 5 days after clinical recovery, seromucoid level in blood serum of turkeys, treated with brovitatoxide, was the same as in clinically healthy poultry (Table. 2).

At research of nonspecific immunity parameters was set, that turkeys infected with eymeriozic invasion, phagocytic activity neutrophils was 15.9% lower, phagocytic index – by 19.7%, phagocytic number – by 22.0% less, compared to clinically healthy poultry (Table. 3).

The obtained results indicate the probable suppression of nonspecific link of immune system (Table. 3).

When applying for the treatment brovitatoxide, the value of FAL, FI, FN on the 3<sup>rd</sup> day were the same as before the treatment.

On the fifth day of treatment it was set clinical recovery of poultry. The indicators of non-specific immunity were increased, but were lower than the control: 12.3% Phagocytic activity neutrophils, Phagocytic index – 13.4%, Phagocytic number – 8.1% (Table 3).

On the fifth day after clinical recovery (the 10th day of the experiment) turkeys state of immune system is not fully returned to normal. Lower compared to clinically

healthy poultry was Phagocytic activity neutrophils – by 8.4%.

The results of the research of brovitatoxide influence when applying collectively with milk thistle fruits on normalization of the immune system in turkeys, affected with eymeriozic invasion are shown in Tables 1–3.

It is established, that because to against eymeriozic action of brovitatoxide and thanks to immune stimulatory action of milk thistle fruits in turkeys which were treated, indicators of cellular and humoral immunity and nonspecific resistance of organism were normalized better and faster, compared to the treatment of one brovitatoxide.

Table 3

**The indicators of nonspecific immunity turkeys, affected with eymeriozic invasion and treated with brovitatoxide (M ± m; n = 20)**

| The indicators<br>Control / Experiment | Day of research              |                              |                             |                             |
|--|------------------------------|------------------------------|-----------------------------|-----------------------------|
|  | The first                    | The third                    | The Fifth                   | The Tenth                   |
| Phagocytic activity<br>neutrophils, %  | 42.3 ± 0.5<br>36.5 ± 0.4**   | 43.3 ± 0.2<br>36.8 ± 0.4**   | 44.6 ± 0.3<br>39.7 ± 0.4*   | 43.8 ± 0.2<br>40.4 ± 0.3*   |
| Phagocytic index, un.                  | 14.6 ± 0.8<br>12.2 ± 0.8**   | 14.5 ± 0.8<br>12.4 ± 0.7**   | 15.2 ± 0.4<br>13.4 ± 0.4*   | 15.2 ± 0.2<br>13.8 ± 0.3*   |
| Phagocytic number, un.                 | 5.32 ± 0.14<br>4.36 ± 0.16** | 5.38 ± 0.24<br>4.47 ± 0.12** | 5.44 ± 0.36<br>5.03 ± 0.16* | 5.46 ± 0.54<br>5.02 ± 0.18* |

At research of cellular immunity indicators in turkeys, treated with brovitatoxide aggregately with milk thistle fruits it was found that for the period of clinical recovery (5<sup>th</sup> day) the number of leukocytes was the same as in clinically healthy (Table 4).

The number of lymphocytes in sick turkeys were 22.7% lower, compared with clinically healthy. In the treatment of their number on the 3<sup>rd</sup> day has increased from 72.2 ± 2.5 g/l to 80.4 ± 2.1 g/l, and on the fifth day was such as in turkeys from the control group.

Table 4

**The indicators cell immunity turkeys, affected with eymeriozic invasion and treated with brovitatoxide and fruit of milk thistle, (M ± m; n = 20)**

| Control / Indicators<br>Experiment | Day of research               |                               |                             |                            |
|------------------------------------|-------------------------------|-------------------------------|-----------------------------|----------------------------|
|                                    | The first                     | The third                     | The Fifth                   | The Tenth                  |
| Leucocytes,<br>G/L                 | 3.45 ± 0.14<br>5.98 ± 0.56*** | 3.54 ± 0.24<br>4.32 ± 0.34**  | 3.42 ± 0.12<br>3.56 ± 0.36  | 3.52 ± 0.14<br>3.48 ± 0.26 |
| Lymphocytes,<br>G/L                | 88.6 ± 2.2<br>72.2 ± 2.1***   | 87.2 ± 2.1<br>80.4 ± 2.5*     | 88.2 ± 1.8<br>86.5 ± 1.7    | 87.4 ± 1.5<br>86.8 ± 1.4   |
| T lymphocytes,<br>(E-RUK) g/l      | 0.12 ± 0.06<br>0.09 ± 0.01*** | 0.12 ± 0.03<br>0.09 ± 0.02*** | 0.11 ± 0.05<br>0.10 ± 0.04* | 0.12 ± 0.04<br>0.12 ± 0.03 |
| B-lymphocytes (EAC-<br>RUK) g/l    | 0.32 ± 0.04<br>0.28 ± 0.04*   | 0.33 ± 0.06<br>0.29 ± 0.06*   | 0.32 ± 0.08<br>0.31 ± 0.06  | 0.33 ± 0.04<br>0.32 ± 0.06 |

Number of T and B lymphocytes on the 3<sup>rd</sup> day of treatment has remained at a low level as well as to treatment, and on the fifth day of treatment was normalized.

On the fifth day after clinical recovery indicators of cellular immunity in turkeys, which were treated with brovitatoxide collectively with milk thistle fruits were the same as in clinically healthy (Table 4).

At research of humoral immunity status in turkeys affected with eymeriozic invasion was set up the decrease of lysozyme activity of serum by 24.5%, bactericidal

activity of serum – 21.6%, and increases in the blood serum level of circulating immune complexes and 33.4% and seromuroid – 88.7%. This points to a significant suppression of humoral immune system.

When applying for the treatment brovitatoxide in aggregate with fruits of milk thistle was found that at the 3<sup>rd</sup> and 5<sup>th</sup> day of lysozyme activity of serum was gradually increased and on 10<sup>th</sup> day reached the control value. Instead BASK was normalized at the fifth day of treatment (Table 5).

Table 5

**The indicators cell immunity turkeys, affected with eymeriozic invasion and treated with brovitatoxide and fruit of milk thistle, (M ± m; n = 20)**

| Indicators<br>Control / Experiment   | Day of research               |                               |                              |                            |
|--------------------------------------|-------------------------------|-------------------------------|------------------------------|----------------------------|
|                                      | The first                     | The third                     | The Fifth                    | The Tenth                  |
| Lysozyme activity of<br>serum, %     | 26.4 ± 1.2<br>21.2 ± 1.4**    | 26.8 ± 1.4<br>22.5 ± 1.6**    | 26.8 ± 1.2<br>23.7 ± 1.5*    | 26.2 ± 1.4<br>25.8 ± 1.7   |
| Bactericidal activity of<br>serum, % | 68.5 ± 2.4<br>56.3 ± 2.6**    | 68.2 ± 1.8<br>58.6 ± 2.7*     | 68.8 ± 2.6<br>65.6 ± 1.8     | 68.8 ± 2.4<br>66.4 ± 1.6   |
| Circulating immune<br>complexes, %   | 25.7 ± 2.3<br>34.3 ± 1.6***   | 25.4 ± 1.4<br>32.7 ± 1.2**    | 25.2 ± 1.2<br>28.6 ± 1.4*    | 25.8 ± 1.4<br>28.4 ± 1.2** |
| Seromuroid mg / cm <sup>3</sup>      | 0.18 ± 0.03<br>0.34 ± 0.05*** | 0.18 ± 0.04<br>0.28 ± 0.03*** | 0.17 ± 0.05<br>0.21 ± 0.04** | 0.18 ± 0.04<br>0.19 ± 0.05 |

In turkeys that were treated, high level of seromucoid in blood serum is gradually decreased, however, on the 3<sup>rd</sup> day was by 55.6% and on the 5<sup>th</sup> day by 23.5% higher than the control. Within 5 days after clinical recovery seromucoid level of in turkeys blood serum, which were treated, was such as in clinically healthy.

The high level of circulating immune complexes in turkeys blood serum, which had been treated, was gradually decreased during the 5-days of the experiment and for the next 5 days after clinical recovery was 10% higher than the control. In analyzing the value of the index of

non-specific immune system was found that the treatment of turkeys, affected with eymeriozic invasion, after applying of brovitatoxide in aggregate of milk thistle fruits phagocytic activity of leukocytes reached normal values at the 10<sup>th</sup> day of the experiment, i.e. 5 days after clinical recovery. Deserves positive assessment by the fact that at the fifth day of treatment were normalized the values of phagocytic index and phagocytic number, which is an indicator of sufficiently high activity of leukocytes (Table 6).

Table 6

**The indicators of nonspecific immunity turkeys, affected with eymeriozic invasion and treated with brovitatoxide and fruit of milk thistle, (M ± m; n = 20)**

| Indicators<br>Control / Experiment | Day of research |             |             |            |
|------------------------------------|-----------------|-------------|-------------|------------|
|                                    | The first       | The third   | The Fifth   | The Tenth  |
| Phagocytic activity                | 42.3 ± 0.5      | 43.3 ± 0.6  | 43.6 ± 0.3  | 43.8 ± 0.2 |
| neutrophils, %                     | 36.5 ± 0.4*     | 38.4 ± 0.3* | 40.7 ± 0.3* | 42.2 ± 0.5 |
| Phagocytic index, un.              | 14.6 ± 0.1      | 14.5 ± 0.6  | 14.2 ± 0.4  | 14.8 ± 0.3 |
|                                    | 12.2 ± 0.8*     | 12.8 ± 0.6* | 13.4 ± 0.4  | 15.0 ± 0.2 |
| Phagocytic number, un              | 5.32 ± 0.1      | 5.68 ± 0.2  | 5.34 ± 0.3  | 5.46 ± 0.5 |
|                                    | 4.36 ± 0.2*     | 4.96 ± 0.3* | 4.82 ± 0.2  | 5.38 ± 0.4 |

Consequently, as a result of the research we found that in turkeys infected with eymeriozic invasion occurs inhibition of cell and humoral immunity and reduces non-specific resistance of organism, leading to the development of secondary immunodeficiency.

**Conclusions**

According to eymeriozic invasion, when applying for treatment brovitatoxide, for a period of clinical recovery, the state of cellular and humoral immunity was significantly improved. But, at 5<sup>th</sup> day after recovery at 20% below normal values was the total number of lymphocytes and at 10% the number of T and B lymphocytes, indicating a partial recovery of the functional state of cellular immunity. The low lysozymic activity of blood serum, and a high level of circulating immune complexes in it at 5<sup>th</sup> day after clinical recovery points to a partial recovery of the functional state of humoral immunity link. The low phagocytic activity of neutrophils confirms it.

The rapid and complete recovery of functional state of immune system in turkeys, affected with eymeriozic invasion was established if brovitatoxide was given aggregately with fruits of milk thistle. The fruits contain group of flavolignans called «Sylimaryn», acting immune stimulating for the development of secondary immune deficiencies state of organism.

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