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INFLUENCE OF PHYTONUTRIENT 'VITASTIM' ON CHICKEN MUCOSAL IMMUNITY AFTER INFECTION WITH LOW-PATHOLOGICAL AVIAN INFLUENZA VIRUS

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Summary. The aim of our study was to investigate the immunostimulatory effect of phytonutrient 'Vitastim' on the immune response after avian infection with low pathogenic avian influenza virus.

It was conducted three groups of experimental chickens of 6 week old (21 birds in each group) to study the mucosal immunity of chickens after experimental infection with low-pathological avian influenza virus, and application of phytonutrient 'Vitastim'. Cryostat sections of 7-μm thickness were prepared, and detection of cells was done using unlabeled primary monoclonal antibodies against the CD4, CD8, IgM, IgG, IgA, macrophage antigens, and a commercially available staining kit. As a negative control, slides were incubated with PBS instead of the monoclonal antibodies. Sections were counterstained with hematoxylin and mounted with Canada balsam. Statistical analysis was performed using SPSS 17.0 software for Windows.

By the results of immunohistochemical research of influence of immunostimulating phytogenous preparation 'Vitastim' on organism of chickens there was determined that given preparation more actively influence on humoral cell of immune reaction in norm and at lowly pathogenic avian influenza (caeca, trachea, lungs) that testified more intensive formation and accumulation of B-lymphocytes which produce immunoglobulins. At research of spleen, there was determined amplified proliferation of T-lymphocytes, macrophages that characterize the activation of cell immune reaction.

The results of immunohistochemical studies of inner organs of chickens of 3 research groups, it was established the influence of the phytonutrient 'Vitastim' on humoral immune system, as evidenced by high levels of IgM, IgG, IgA. 'Vitastim' had stimulating impact on state of cell immunity, however, as evidenced by the low levels of CD4, macrophages, in chickens of experimental groups compared to the intact birds, cell immunity does not play a significant role in the pathogenesis of LPAI.

Keywords: avian influenza virus, cell-, and humoral-mediated immune response, immunohistochemistry, low pathogenic avian influenza

Introduction. Poultry production now is rapidly developing and needs of modern veterinary support. The presence of accelerated evolutionary processes resulted in a complication of the epizootic situation, increasing the pathogenic properties of the pathogens, the spread of infectious diseases.

Way out lies in enhancing the natural resistance of the organism, strengthen the immune status at the level of the organism and group immunity by eliminating the immunosuppressive factors and use immunostimulatory means. Immunostimulation is widely used in infectious disease. There is used the adjuvants of different origin. They are valuable way to improve the immune status of the avian organism and enhance the immune response during vaccination. Search for new immunostimulating preparations continues to view ever-increasing requirements regarding their safety, effectiveness, and accessibility.

Recently, considerable attention is paid to herbal preparations (Lee et al., 2008, 2011). Many plants are well known in ethnoscience and have pronounced immunostimulatory properties. *Echinacea purpurea* has such properties. The root alcoholic extract is prepared from this plant (Kogut and Klasing, 2009).

Herbal immunostimulants also prepared by combining several plants in view of the focus of their actions on the organism. Such immunostimulant significantly increases the level of antibodies in vaccinated birds (Lee et al., 2010). Phytonutrient 'Vitastim' showed a high immunostimulating activity and harmlessness at using for chickens under experimental conditions (Krasnikov et al., 2002).

Materials and methods. *Experimental animals.* It was used 63 6 week-old chickens to study mucosal immunity state after application of phytonutrient 'Vitastim'. It was formed 3 groups from them:

1. Chickens experimentally infected with law pathogenic avian influenza virus A/mallard/Ukraine/2007 H5N2 intraintranasally in the dose 10^3 EID_{50} per chicken, and given phytonutritin 'Vitastim' in the doze of 2 mg/kg;

2. Chickens, which were given phytonutritin 'Vitastim' in the doze of 2 mg/kg;

3. Control untreated chickens.

Water and feed were provided *ad libitum*.

Immunohistochemistry. Pathological material was collected on 1st, 3rd, 5th, 7th, 10th, 14th, and 21st days post infection. Than it was prepared histological sections from spleen, caeca, trachea, lung fixed in liquid nitrogene. Cryostat sections of 7-μm thickness were prepared, and detection of cells was done using unlabeled primary monoclonal antibodies against the CD4, CD8, IgM, IgG, IgA, macrophage antigens, (Southern Biotechnology Associates, Eching, Germany) and a commercially available staining kit (LSAB, ChemMate Detection kit, peroxidase antiperoxidase, rabbit/mouse; DakoCytomation, Hamburg, Germany). As a negative control, slides were incubated with PBS instead of the monoclonal antibodies. Sections were counterstained with hematoxylin and mounted with Canadian balsam (Riedel de Haen AG, Seelze-Hannover, Germany).

Statistical analysis. Statistical analysis was performed using SPSS 17.0 software for Windows. All data for each group expressed as means ± SEM. The difference between the means considered at $p < 0.05$.

Results. *Immunohistochemistry.* With the purpose of immunohistochemical research of forming of immune answer of chickens' monoclonal antibodies were applied to subpopulations of immunocompetent cells: CD4, CD8, IgM, IgG, IgA, macrophages and M-cells.

At the detailed study of dynamics of forming of immune answer in birds infected by the virus of lowly pathogenic influenza + phytonutrient 'Vitastim' (the 1st group), in intact birds, which got the phytonutrient 'Vitastim' (the 2nd group), and control birds (the 3rd group) the next changes of amount of immunocompetent cells were detected in inner organs.

Therefore, in a spleen at investigation of cells CD4 observed the increase of these cells already on the 3rd day of experiment (26.930 ± 0.767%) against 18.193 ± 4.602% — on the 1st day of experiments. Process of increase lasted to 7th day, when the amount of cells acquired maximal values (22.153 ± 0.378% against 18.213 ± 0.431% — in a control bird). Maximal indexes in chickens of the 1st experimental group the amount of cells after period of suppression attained on 10th day (21.03 ± 0.421% at 18.213 ± 0.751% — in control). Moreover, the level of CD4 on 10th–21st days of experiments was higher than in chickens of the 2nd and the 3rd groups. There has been noticed that in an intact bird the percent amount of cells-helpers decreased since 7th day (20.943 ± 2.047%) and to the end of term of observation on 21st day (13.056 ± 0.246%). The fact of increase of subpopulation of cells CD4 in the chickens of the 2nd group on a background the reduction of amount in a control bird testifies immunostimulating influence of preparation 'Vitastim' (Fig. 1).

At the study of subpopulation of lymphocytes with the superficial marker of CD8 a considerable increase of the percent of these cells in experimental and control birds. As in the case with CD4, the amount of CD8 began to increase on 3rd day, thus its amount in experimental birds was far above, than in control. So, on 3rd day in chickens of the 2nd group the amount of CD8 was 37.246 ± 0.763% against 21.503 ± 3.579% — in a control bird. Starting with 5th day the percent amount of CD8 began to decrease. However in the experimental bird of the 2nd group during two weeks after the task of preparation observed a trend to the increase of amount of these cells with a maximal index on 10th day — 28.933 ± 4.065% against 26.380 ± 0.575% — in an intact bird. Maximal indexes in chickens of the 1st experimental group the amount of cells attained on the 7th day with index 29.136 ± 0.604% at 28.836 ± 0.759% — in control. The level of CD8 in chickens of the 1st group on 10th–21st days of experiments was higher than in chickens of the 2nd and the 3rd experimental groups.

Content of macrophages in a spleen was characterized by a reliable increase from first day of observation (8.936 ± 1.843% on 3rd day against 7.146 ± 1.023% on 1st day of investigations in chickens of the 2nd group. The level of macrophages differed low difference between the experimental and control bird but it was higher in chickens of the 1st group with the maximal index on the 7th day 11.463 ± 0.419. Process of reliable increase of amount of this subpopulation of cells in chickens of the 2nd group lasted to 7th day and attained to 11.443 ± 0.399% against 10.153 ± 0.580% in the group of control. The percent amount of macrophages decreased in future but was higher than on 1st day of investigations (7.033 ± 0.431% in the 1st group against 6.378 ± 1.452 — in intact chickens.

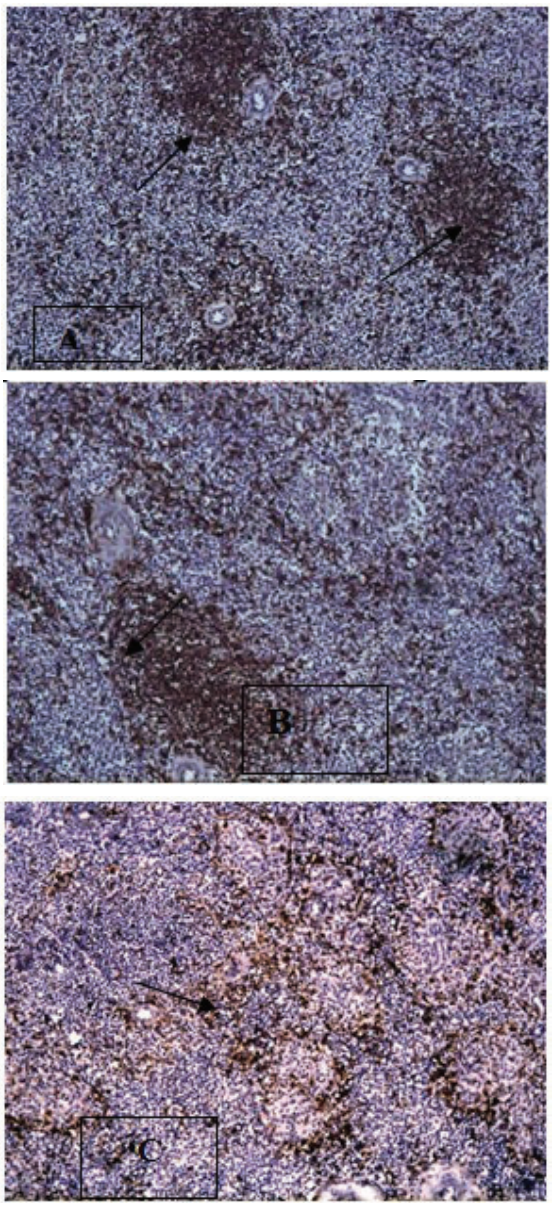

Figure 1. CD4 — aggregates in chicken spleen on 5th day after infection with LPAIV + phytonutrient 'Vitastim' (A), phytonutrient 'Vitastim' feeding (B), and in control chickens (C). Arrows show on cell aggregates in the form of conglomerates and dots of brown colour. It is also shown that the number of cells in the spleen of chickens of 1st and 2nd groups (A, B) prevail over the control (C). LSAB-method, × 200.

An amount of IgM-expressing cells in the first three days of observation was considerably greater in chickens of the 2nd group, than in the 1st group and control. Therefore, on 1st day their percent amount presented 3.566 ± 0.405% against 3.45 ± 0.900% in the control group. In addition, the process of reliable increase of amount of these cells was fixed in a spleen with a maximal index on 10th day, which in chickens of the 2nd group presented 3.653 ± 1.888% at 2.010 ± 1.168% in the group of intact chickens. In chickens of the

1st experimental group this index was higher than in chickens of the 2nd and 3rd groups and presented 4.193 ± 0.433%. Than the percent of these cells began to decrease but was considerably greater in the group of infected chickens, which got 'Vitastim'

At the immunohistochemical study of content of IgG-expressing cells in the histological sections of spleen was not carried out the tendency to the gradual increase or decrease of percent amount of these cells, only oscillation in ether, one or another side. But starting with 10th day of observations we are carried out the increase of amount of cells of this subpopulation in chickens of the 2nd group from 6.240 ± 2.124% at 3.710 ± 1.211% — in the control group that lasted to the end of term of observations on 21st day, when the amount of these cells got the highest value (8.546 ± 0.452% at 5.951 ± 0.521% — in the group of intact chickens. But from 10th day the amount of cells in chickens of the 1st group begun considerably increase and was higher than in the 2nd and in the 3rd groups to the end of term of observations attaining the maximal index 9.07 ± 0.191%. The process of the percent of cells IgG was with decrease of IgM percent. Possibly, it is connected with change of protective functional from IgM to IgG (Fig. 2, 3).

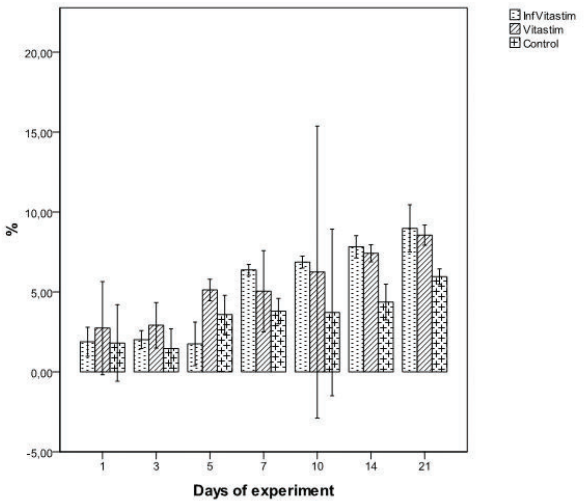

Figure 2. Dynamics of IgG changes in chicken spleen after phytonutrient 'Vitastim' feeding. Each bar represents the accumulation of cells in the immune process dynamics since 1st to 21st days after phytonutrient administration. Significant stimulating influence of phytonutrient 'Vitastim' on immune response against LPAI is shown since 7th day. Statistical analysis was performed by comparing cell amount in spleen of chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim), phytonutrient 'Vitastim' feeding (Vitastim) with control birds.

At the investigation of caeca there was determined gradual increase of cells with superficial marker CD4 from 1st day (4.826 ± 0.337% at 2.950 ± 0.265% in control) to 5th day (6.756 ± 0.241% at 4.32 ± 0.536% in (intact) chickens of the 2nd group). But in chickens of the 1st group after low suppression on 3rd–5th days was determined the increase of amount of cells on 10th day with maximal index 24.456 ± 2.249%. Moreover, the level of cells in chickens of the 1st group was far above during all period of observations. After that in chickens of the 2nd group starting with 7th day the process of activation of immune system became sharp character when the indexes on 7th and 14th days were 9.823 ± 1.289% and 14.726 ± 0.534%. The amount of cells-helpers in chickens of the 2nd group significantly overstated the amount in control group in this interval (5.980 ± 0.420% and 7.090 ± 0.725% on 7th and 14th days respectively). On 21st day the percent content CD4 decreased but significantly overstated the indexes on 1st day of investigations.

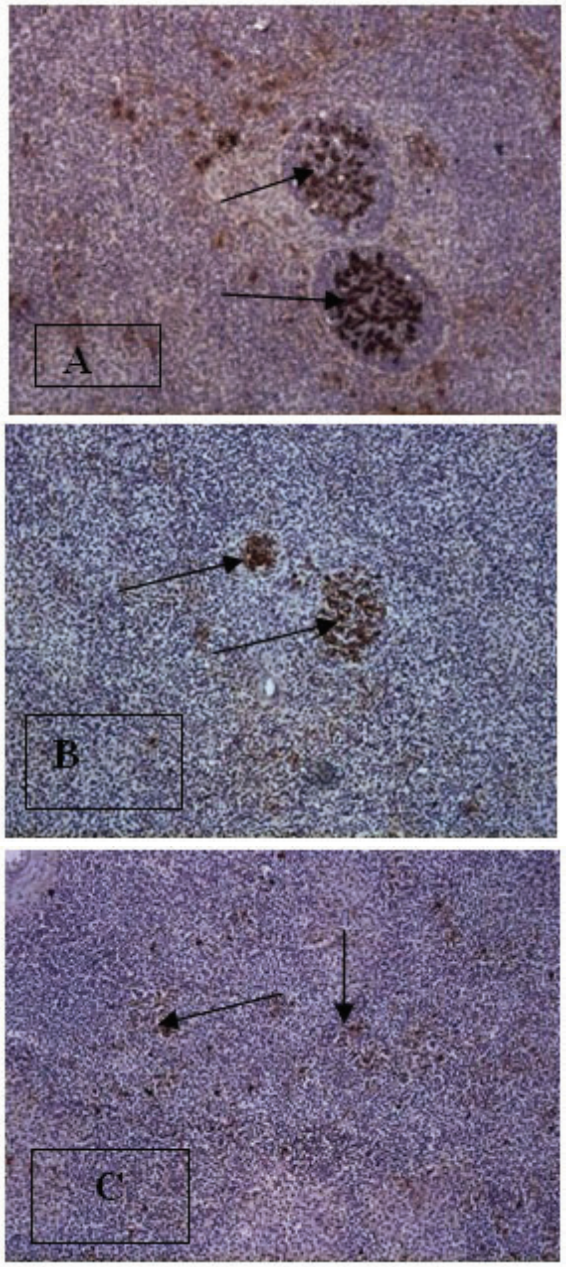

Figure 3. IgG aggregates in chicken spleen on 14th day after infection with LPAIV + phytonutrient 'Vitastim' (A), phytonutrient 'Vitastim' feeding (B), and in control chickens (C). Arrows show on cell aggregates in the form of conglomerates and dots of brown colour. It is shown an immunostimulative influence of phytonutrient 'Vitastim' that proved by intensive accumulation of IgG in germinative lymphoid follicles (A, B) as compared with control (C). LSAB-method, × 200.

The dynamics of accumulation of cells with superficial marker CD8 in chickens of the 2nd group was characterized by the insignificant, gradual increase of percent amount from 1st (11.716 ± 2.855%

at 4.853 ± 1.834% in the group of control chickens) to 10th day inclusive (15.356 ± 3.933% at 10.763 ± 1.168% in control). Maximal values the percent of cells attained on 14th day — 19.833 ± 0.245% at 12.143 ± 0.149% in intact chickens, after that there was an insignificant decrease of activity of these cells, which however was higher than in the previous terms of research. As for chickens of the 1st group during the period of suppression from 1st to 5th days the level of these cells was far above than in other groups of chickens. So on 1st day the level of CD8 in chickens of the 2nd group presented 20.28 ± 0.384% 4.853 ± 1.834% in the group of control chickens. On 14th day the level of cells begun decreased and the indexes were lowest than the level of chickens of the 2nd and the 3rd groups.

Chickens which got preparation 'Vitastim' (the 2nd group) characterized by getting up of functional activity from 3rd day (1.866 ± 0.190% at 2.343 ± 0.669% in control) to 10th day inclusive with a maximal index 4.063 ± 0.193% at 2.833 ± 0.162% in intact birds have an amount of IgM-expressing cells. In further observed the period of slump of activity to the end of term of observations on 21st (2.178 ± 0.184% at 1.896 ± 0.254% in the group of control). In chickens of the 1st experimental group the indexes during all period of research were less than in chickens of the 2nd and the 3rd groups with maximal level on 2.226 ± 0.585% — on 10th day of observations.

These data correlated with data of changes of the content of IgG-expressing cells. So, in a period, when observed the rise of activity of IgM (that is from 1st to 10th days of research), the level of IgG hesitated towards to the increase and decrease of activity. However, on 10th day, when the percent amount of IgM decreased, there was an activation of making of IgG. So, on 10th day of observation in chickens of the 2nd group its content presented 6.910 ± 0.026% at 3.113 ± 0.239% in control chickens. The process of functional activity rise of these cells in course of time acquired sharp character and arrived at maximal values on 21st day of experiment (7.012 ± 0.237% at 3.894 ± 0.219% in the 2nd group of intact chickens. But in chickens of the 1st group the process of increase of percent amount attained more sharp character than in chickens of the 2nd and the 3rd groups with maximal index 9.983 ± 0.128% on 21st day of observations.

At research of dynamics of IgA-expressing cells did not observe a clear dynamics to the increase or decrease of percent amount of these cells. Moreover, the level of IgA in chickens of the 2nd group did not almost differ from indexes of control chickens (3.960 ± 0.310% at 3.486 ± 0.244% in intact bird on 5th day of research). Only beginning from 14th day of observations in chickens of the 2nd group the clear increase of content of cells was specify with a marker to 4.533 ± 0.538% at 3.826 ± 0.615% in control that lasted to the end of term of observations on 21st day and presented 6.146 ± 0.394% at 5.124 ± 0.407% — in an intact bird. However, as in previous case the level of cells in chickens of the 1st group during 7th–21st days was considerable higher than in chickens of the 2nd and the 3rd groups with maximal index 12.923 ± 0.334% on 21st day of studying (Fig. 4).

Such reliable increase of IgA can testify about positive influence of applied immunostimulating phytonutrient 'Vitastim' on the state of local immunity which takes place on the mucous membranes of respiratory and gastrointestinal tracts.

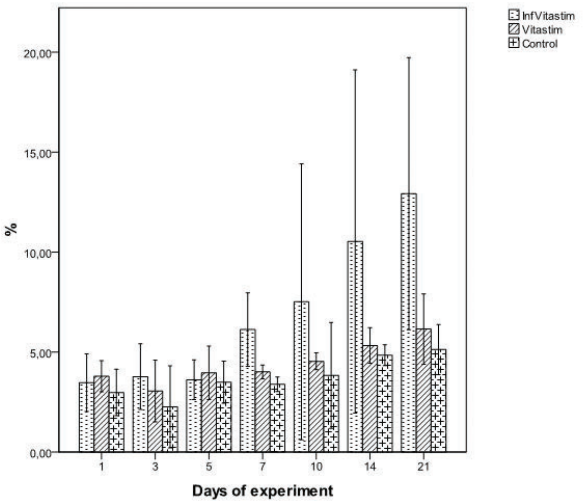

Figure 4. Dynamics of IgA changes in chicken caeca after after phytonutrient 'Vitastim' feeding. Each bar represents the accumulation of cells in the immune process dynamics since 1st to 21st days after phytonutrient administration. Significant stimulating influence of phytonutrient 'Vitastim' on immune response against LPAI (especially in chickens infected with LPAIV + phytonutrient 'Vitastim') is shown since 7th day. Statistical analysis was performed by comparing cell amount in caeca of chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim), phytonutrient 'Vitastim' feeding (Vitastim) with control birds.

During CD4 examination in lungs the sharp increase of their activity was established almost in two times in the 1st group of chickens which got preparation 'Vitastim'. Therefore, on 3rd day the number of CD4 was 4.170 ± 0.205% at 2.416 ± 0.335% in control. However, on 7th day in the group of control there was observed the sharp increase of cells to 4.026 ± 0.123%. Thus, phytonutrient 'Vitastim' stimulates activation of process of accumulation of CD8 earlier. Stimulation of immune answer took place in the chickens of the 2nd group to 14th day of study, when the content of killers acquired maximal numbers and was 5.920 ± 0.703% against 3.676 ± 0.182% in control. As for the group of intact chickens, then a tendency to the gradual decrease of activity of CD4 was observed already on 10th day (3.930 ± 0.472%) and lasted to the end of term of observations on 21st day. However in chickens of the 1st experimental group after period of suppression on 1st–5th days of experiments observed the period of sharp rise of activity of these cells on 7th day, moreover their amount on 7th–10th days was far above than in chickens of the 2nd and the 3rd groups and presented 6.683 ± 0.576% on 10th day. After that was observed percent decrease of these cells but their amount was higher than in control.

CD8 had similar dynamics with CD4. Therefore, the insignificant increase of cells in relation to control was observed in chickens of the 2nd group from the 3rd day (3.963 ± 0.977% at 2.976 ± 0.261% in the group of control) and lasted to 5th day with an index 4.863 ± 0.933% at 4.436 ± 0.449% in the group of intact birds. However, from 5th day the process of increase of cell activity acquired sharp character and presented 7.410 ± 0.266% at 5.243 ± 0.222% in the chickens of control group. The maximal reliable indexes of CD8 were in the chickens of the 2nd group on 10th day (8.276 ± 0.250% at 5.080 ± 0.540% — in the group of intact chickens). On 14th day and to the end of term of observations occurred very slow decrease of the

percent account of CD8 as opposed to the control chickens, where the activity decrease was significant on 21st day (3.214 ± 0.813% at 5.173 ± 0.200 — on 14th day of research). Dynamics of changes of CD4 was almost similar in chickens of the 1st experimental group. During the first ten days of observations, their amount was higher than in chickens of the 2nd and the 3rd groups The subpopulation has maximal indexes on 10th day — 3.88 ± 0.576%. After that, there was observed the percent decrease of these cells but their amount was higher than in control.

Indexes of macrophages in lungs were considerably less, than in spleen, but the character of accumulation almost not differed (Fig. 5).

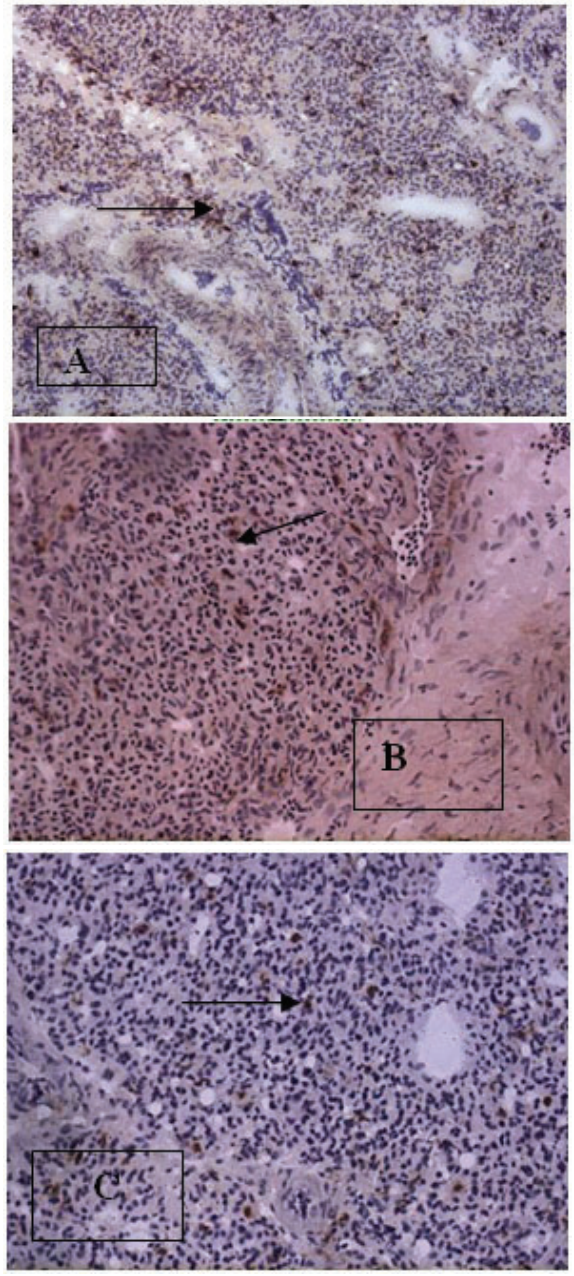

Figure. 5. Macrophage aggregates in chicken lung on 3rd day after infection with LPAIV + phytonutrient 'Vitastim' (A), phytonutrient 'Vitastim' feeding (B), and in control chickens (C). Arrows show on cell aggregates in the form of brown coloured dots. It is shown small ammount of these cells in the lung of chickens of all experimental groups during period of observation. LSAB-method, × 200.

Therefore, since first time of observations it was determined the tendency to the gradual increase of percent amount of macrophages, as in the group of chickens, which got phytonutrient 'Vitastim', and in control, intact birds. But if in control group sometimes insignificant oscillation of amount of cells has been observed toward decrease or increase then in chickens of the 2nd experimental group the process of activation of accumulation of macrophages had a clear tendency to the increase to 5th day of research, when the level of cells acquired maximal index 6.793 ± 0.473% and 5.663 ± 0.342% (in chickens of the 1st and the 2nd groups) at 3.7 ± 0.365% in intact birds. After that from 7th day the insignificant slump of activity of macrophages, that lasted to the end of term of observations on 21st day and presented 4.296 ± 0.467% and 4.264 ± 0.452% (in chickens of the 1st and the 2nd groups) at 3.347 ± 0.512% — in control.

At research of IgG-expressing cells is determined its insignificant quantity in the first five days of observations. However, on 7th day in chickens of the 2nd group was a sharp insignificant increase of the percent of their cells on a background of sharp decrease of IgM-expressing cells (5.653 ± 0.139% at 4.113 ± 0.133% in an intact bird) but their level in control group was considerably below during 7th–21st days after treatment (6.532 ± 0.235% at 4.540 ± 0.214% in control at the end of study). A sharp increase of IgG-expressing cells in chickens of the 1st group occurred on 10th day (4.18 ± 0.407% at 4.046 ± 0.278% — in control). In future there was observed the percent increase of these cells in chickens of the 1st group with maximal index 5.643 ± 0.238% on 21st day of research, moreover their level was higher than control during all period of observations.

The vibrations of percent amount of cells in the side of increase or decrease observed at the study of subpopulation of IgA-expressing cells. A clear tendency to the increase to the percent of cells was marked from 10th day of research (3.946 ± 0.315% and 3.023 ± 0.800% in chickens of 1st and 2nd groups at 1.846 ± 0.290% in intact chickens). The increase of activity of cells lasted to the end of term of observations on 21st day and acquired a maximal level with an index 6.063 ± 0.239% and 5.214 ± 0.124% at 2.412 ± 0.135% in the group of control chickens. Moreover during all period of observations the amount of cells in chickens of 1st group was greater than in chickens of 2nd and 3rd groups (Fig. 6).

In trachea when studying CD4 watched for an insignificant trend to the increase of percent amount of these cells in the chickens of the 2nd group during the first five days. So, on 5th day the content of CD4 presented 1.516 ± 0.318% against 0.883 ± 0.141% in the control group of chickens. Starting with 7th day there was a considerable increase (almost in two times) of cells of this subpopulation, attaining the maximal indexes on 10th day (2.743 ± 0.254% — in chickens of the 2nd group at 1.876 ± 0.812% in an intact bird). In further there was a process of rapid slump of activity of these cells, which lasted to the end of term of observations on 21st day (1.856 ± 0.121% — in chickens of the 2nd group at 0.964 ± 0.136% in the chickens of control group). As for chickens of the 1st group that curve of changes of accumulation dynamics characterized by considerable amount of cells concerning to chickens of the 2nd and the 3rd groups during all period of observations (almost in three times greater). So on 1st day the amount of cells presented 3.41 ± 0.617% at 1.033 ± 0.224% and 0.526 ± 0.255% — in chickens of the 2nd and the 3rd groups. Cells with markers CD4 attained the maximal index on 10th day (4.686 ± 0.282%). On 14th–21st days of observations in chickens of the 1st group occurred the process of slow decrease of cell amount.

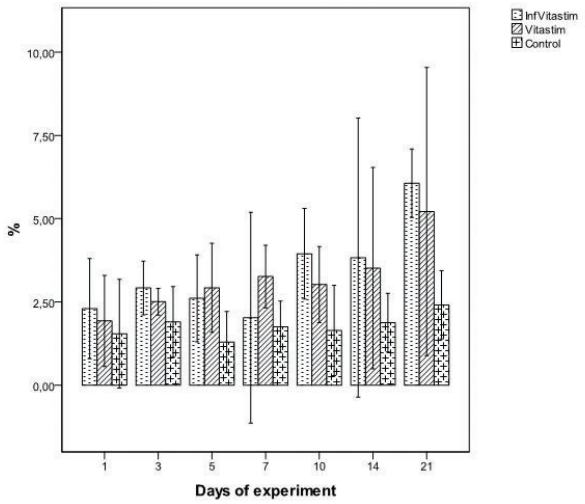

Figure 6. Dynamics of IgA changes in chicken lungs after phytonutrient 'Vitastim' feeding. Each bar represents the accumulation of cells in the immune process dynamics since 1st to 21st days after phytonutrient administration. Significant stimulating influence of phytonutrient 'Vitastim' on immune response against LPAI is shown since 3rd day. Statistical analysis was performed by comparing cell amount in lung of chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim), phytonutrient 'Vitastim' feeding (Vitastim) with control birds.

At the study, CD8 it is registered its significant amount in chickens of the 1st group comparatively with the 2nd group and the group of control more than in three times during all period of study. During all time there was a clear tendency to the increase of percent amount of these cells. Maximal indexes of CD8 attained on the 10th day of research, when its level presented in chickens of the 1st group — 5.13 ± 0.461%, in the 2nd group of chickens 2.216 ± 0.708% at 1.166 ± 0.156 in the group of control bird. In a further period of research observed the insignificant decrease of amount of these cells to the end of term of experiments on the 21st day (Fig. 7).

When studying IgG in chickens of the 2nd group is determined its insignificant amount in the first seven days of observations (0.396 ± 0.044% at 0.196 ± 0.042% in the bird of control group. Exactly on 10th day a sharp reliable increase of percent of their cells was marked on a background of the decrease of IgM-expressing cells (1.770 ± 0.183% at 0.343 ± 0.117% — in intact chickens. An increase lasted to ends of term of observations on 21st day, when an index acquired maximal values — 6.542 ± 0.162% at 0.984 ± 0416% — in the control group of chickens. In chickens of the 1st group there was also determined insignificant its amount in first seven days of observations (0.573 ± 0.078%). but on 10th day the amount of cells considerably grew and exceeded levels in chickens of the 2nd and the 3rd groups with maximal index on 21st day 3.72 ± 0.190% (Fig. 8).

A clear tendency to the IgA increasing to the percent of cells was marked from 10th day of research (2.09 ± 0.175% and 1.640 ± 0.160% in chickens of the 1st and the 2nd groups at 1.076 ± 0.218% in control bird) and to the end of term of observations with a maximal level on 21st day — 2.876 ± 0.518% and 2.312 ± 0.185% in chickens of the 1st and the 2nd groups at 1.554 ± 0.156% in group of intact chickens. As in previous case the amount of cells in birds of the 1st group was far above than in chickens of the 2nd and the 3rd groups.

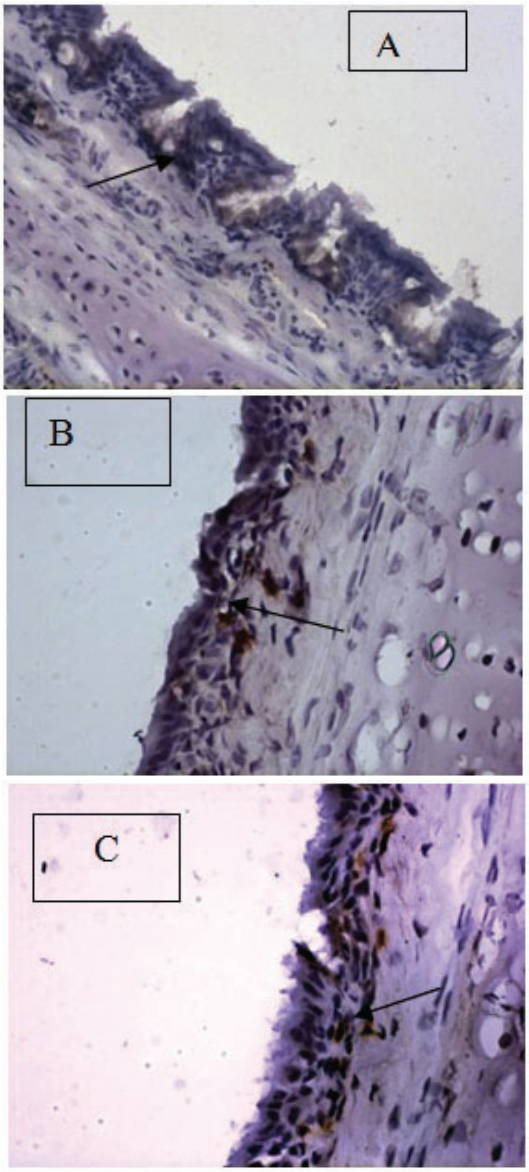

Figure 7. CD7 aggregates in chicken trachea on 3rd day after infection with LPAIV + phytonutrient 'Vitastim' (A), phytonutrient 'Vitastim' feeding (B), and in control chickens (C). Arrows show on cell aggregates in the form of brown coloured dots. It is shown small ammount of these cells in the traches of chickens of all experimental groups during period of observation. LSAB-method, × 200.

Conclusion. Data of realized research certify that character of cell amount changes of different subpopulations in a spleen, lungs, caeca and trachea of chickens of 1st, 2nd and 3rd groups is not differed substantially. However, there are existed some differences.

Differences testified that in a spleen (comparatively with first day of research) on 1st–10th days took place sharp increase of amount of CD8, macrophages. On 10th–14th days there was an increase of amount of IgG and IgA at some diminishing of content of cells with the markers of macrophages and IgG comparatively with 7th day of research. On 14th–21st days the certain stabilization of content of clusters was marked at level, near to their value on 10th day.

Content of IgA-expressing cells notedly grew short in a period between 1st and 7th days and rose in future, arriving at a maximum on 21st day.

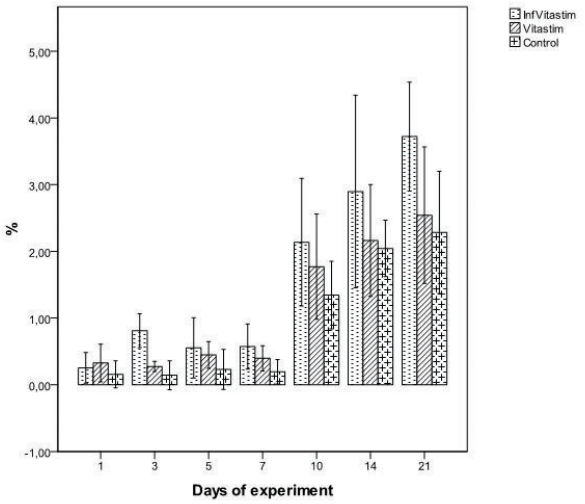

Figure 8. Dynamics of IgG changes in chicken trachea after phytonutrient 'Vitastim' feeding. Each bar represents the accumulation of cells in the immune process dynamics since 1st to 21st days after phytonutrient administration. Significant stimulating influence of phytonutrient 'Vitastim' on immune response against LPAI (especially in chickens infected with LPAIV + phytonutrient 'Vitastim') is shown since 10th day. Statistical analysis was performed by comparing cell amount in lung of chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim), phytonutrient 'Vitastim' feeding (Vitastim) with control birds.

In lungs paid attention on itself higher indexes of content of IgG and IgA (almost in two times) in chickens of group 1 and 2 in relation to chickens of control group, beginning from 10th day. At the same time on 10th day observed sharp diminishing of IgM-expressing cells, which lasted to the end of term of observation on 21st day what testifies about the transmission of function of protection from IgM to IgG on it stage of immune process. At research of content of CD4 and CD8 observed a trend to the increase of these cells of 7th for 14th days of research at decrease of them in intact chickens, that can testify about immunostimulating influence of preparation 'Vitastim' on the immune state of experimental bird.

In a caeca observed the sharp increase of CD4, since 10th day especially in chickens of 1st group in relation to control chickens. The process of increase lasted to (the end of term of observations) 10th day. Amount composition of these cells prevailed over the amount of CD4 in lungs that can testify about more considerable role of this organ in forming of immune reaction. Moreover, the amount of macrophages in all investigational organs considerably grew already in the first terms of research that testified that they get the first in the process of immune reaction.

In the trachea of intact chickens, which got preparation 'Vitastim' and chickens of 1st group observed the considerable predominating of cells with markers of CD8, IgM above the level of these cells in a control bird. On the end of term of observations looked after the maximal level of IgG, which was greater than control bird in two times. Immunohistochemical determination of the amount of cells with the marker IgG can be the criterion of determination of activity of immunostimulating preparations. There were less CD4 than in other investigational organs during all period of observations.

By the results of immunohistochemical research of influence of immunostimulating phytonutrient 'Vitastim' on organism of chickens there was determined that given preparation more actively influence on humoral cell of immune reaction in norm and at lowly pathogenic avian influenza (caeca, trachea, lungs) that testified more intensive formation and accumulation of B-lymphocytes which produce immunoglobulins. At research of spleen, there was determined amplified proliferation of T-lymphocytes, macrophages that characterize the activation of cell immune reaction.

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