Non-specific resistance and specific immunogenesis of the body of the bird cross Loman Brown on the background of biostimulation

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Abstract. PV-1 immunostimulant influence on the parameters of non-specific resistance and specific immunogenesis of bird cross Loman Brown is discussed in the paper. It was established experimentally that feeding chickens by the PV-1 preparation in doses of 0.05 ml/kg, 0.10 and 0.15 ml/kg of body weight, respectively, once a day within 10 days with a 10-day break, with repeating cycles up to 111-day of their age, stimulates the growth and development of young. Immunization of a bird against the background of the use of the immunotropic drug PV-1 is accompanied by an increase in immunity: when chickens are vaccinated against Gumboro disease, the titers of specific antibodies are increased by 53.3%, against Newcastle's disease - by 50.0% and against egg drop syndrome-76 - by 26.5%. An increase in the egg productivity of laying hens grown on the background of the use of PV-1 was established: egg production for the initial hen increased by 9.2 - 17.8%, egg weight - by 0.5 - 4.6%. Studying the meat productivity of poultry grown on the background of the use of the PV-1 preparation, an increase in the slaughter yield of 1.7–5.0% was established, the yield of edible parts increased by 6 ±3.20 – 155±3.40 g (P <0.001).

1. Introduction

The poultry industry has gained significance all over the world. In 2012, the FAO (Food and Agriculture Organization) estimated 103.5 million tons of annual global chicken meat production which contributed about 34.3% to global meat production [1]. Among food animals, chicken meat and eggs are the most efficient protein sources. Overall, poultry farming has performed a leading role in the livestock sector in some parts of the world [2]. Chickens are providing economical, healthier food than red meat and other protein sources [3].

The poultry industry currently accounts for the production of around 118 million metric tons of meat and around 74 million metric tons of eggs annually. As the global population continues to increase, so does our reliance on poultry as a food source. It is therefore of vital importance that we...
safeguard this valuable resource and make the industry as economically competitive as possible. Avian viral infections, however, continue to cost the poultry industry billions of dollars annually [4].

In the last decades poultry meat and egg consumption increased strongly worldwide. The reasons for this increase are manifold but the main factor for the increasing poultry meat demand is that it represents a cheap animal protein source [5, 6]. Although, in the past, poultry were bred and reared at the farm level, today a few globally operating breeding companies provide the birds to specialized farms. This was associated with a tremendous specialization in the poultry sector, which resulted in the 2 decoupled branches of production, egg and meat, with correspondingly specialized breeding lines [7].

In providing the population with quality food a special place is given to the poultry industry, which is able to solve this problem in a short time and at the lowest cost. However, it must be remembered that further development and improvement of the competitiveness of the poultry industry is possible only with the widespread introduction of innovative resource-saving technologies and equipment to maximize the genetic potential of bird productivity [8-10].

High levels of production in intensive farming systems are associated with increased replacement rates as a result of multifactorial diseases. The so-called "production diseases" may include low-grade infection reducing profitability without increased morbidity. Such infections are sustained by low pathogenic viral and bacterial agents which give rise to full-blown disease in association with poor environmental conditions. In these farms, the results of vaccination may be disappointing. Therefore, fundamental issues should be dealt with toward successful immunoprophylaxis. A negative modulation of the host microbiome by farm management practices and drug treatments is a further risk factor. The immune response to stressed cells questions the usual correlates of protection investigated after vaccination. In particular, there is evidence that specific and non-specific immune responses may overlap in vitro as a result of a high level of innate immune responses to Damage-Associated Molecular Patterns (DAMPS) and stress antigens. A vigorous adaptive immune response to microbial agents may be sometimes counterproductive [11].

Variations of environmental factors such as sun light, temperature, humidity and characteristics of animal metabolism and the mechanism of thermo-regulation can cause imbalances in the animal body [12, 13]. An increase in temperature might influence the susceptibility of pathogens (bacteria and parasites) in the environment of chickens. Heat stress negatively effects the plant growth (cereal grains) and causes poor feed quality which can affect the poultry growth rate (daily weight gain) due to reduce feed efficiency [14, 15]. Ayo et al. [16] described that layer chickens had a 20% reduced feed intake during hot and humid weather. The above authors recorded a decrease in egg production and a drop in hen-day production.

In the industrial poultry industry, achieving high productivity, safety and obtaining biologically complete and benign products is sometimes very problematic, due to the pressure of environmental and technological factors of the environment, which negatively affects the physiological state of the body. Under the influence of these adverse factors, the nonspecific resistance and immunological reactivity of the bird are often reduced. Damage to the immune system leads to an immunodeficiency state and weakening of the body's resistance to infectious agents [17-19].

The world's direction in the past decades was focusing on the usage of growth promoters for improving the productive performance; therefore, antibiotics were used extensively in poultry industry as growth promoters. The results of using antibiotics were magnificent in increasing the performance traits, but they contributed the development of antibiotics-resistant. There is a great interest in developing natural alternatives for the growth promoters [20].

The use of antibiotics and chemotherapeutic agents for the prevention and treatment of poultry diseases often leads to disruption of normal microflora, the emergence of resistant strains of pathogens and a decrease in immune status. Creating healthy livestock through the introduction of science, technology, and best practices will further enhance the productivity of poultry [21, 22].

To date, numerous studies are focused on searching for alternatives to antibiotics with similar antimicrobial and growth-stimulating effects that do not cause bacterial resistance and potential side
effects for animals [23].

In the context of the above, the problem of restoring immunological disorders using immunostimulants is currently relevant to modern science and practice, since most diseases are accompanied by secondary immunological failure [3, 8, 10]. The purpose of this work is the realization of the biopotential of the bird by the correction of non-specific resistance and specific immunogenesis of the organism.

2. Material and methods

The experimental part of the research work was carried out at one of the poultry farms of the Chuvash Republic, and the materials were processed in the laboratory of bio- and nanotechnologies of the FSBEI HE Chuvash State Agricultural Academy. The objects of research were the clinically healthy bird of the Lohmann Brown autosexing cross-country egg direction. Four groups of day-old chickens of 60 animals each were formed according to the principle of analog groups. Chickens of the 1st experimental group were fed with PV-1 immunostimulant in a dose of 0.05 ml/kg body weight, the 2nd experimental group - 0.10 ml/kg, and the 3rd experimental group - 0.15 ml/kg of weight body. The drug PV-1 was fed with feed once a day for 10 days with a 10-day break, with repeating cycles up to 111-day-old birds. The control group of chickens did not receive the drug. The young of experimental groups up to 111-days-old were kept in the growing shop and then transferred to the shop for keeping laying hens of the parent flock. The conditions of housing, feeding, and care for all groups of birds were the same.

PV-1 – developed by employees of the Chuvash State Agricultural and Economic Academy (F.P. Petryankin, N.K. Kirillov, V.G. Semenov). It is a suspension containing antiseptic Dorogov stimulus-torus - ASD (F-2), vitamins (ascorbic and para-aminobenzoic acids), hydrochloric acid and formalin. It has a specific smell and color - from light yellow to reddish brown, easily dissolves in water, does not mix with oils and organic solvents, is resistant to heating and cooling. PV-1 has a pronounced biological effect to activate the T- and B-lymphocyte and macrophage systems. The preparation is able to increase phagocytosis, increase the level of lysozyme activity of plasma and bactericidal activity of blood serum, the total amount of protein and its γ-globulin fraction in blood, resist diseases. The preparation improves tissue trophics, normalizes metabolic processes in animals in various dystrophic states [24]. PV-1 is approved by Department of veterinary science of the Ministry of Agriculture of the Russian Federation of 25.09.01 No. 13-4-03/0193.

Number of erythrocytes, concentration of hemoglobin, total number of leukocytes were determined on the Coulter LH 750 Beckman Coulter automatic hematology analyzer (USA). Analyzer allows to carry out blood examination according to 31 parameters, including differentiation of leukocytes according to 5 populations.

The total protein level and protein spectrum in serum were determined on the IDEXX VetTest 8008 biochemical analyzer (IDEXX, Russia). The VetTest analyzer offers to perform a series of steps, accompanying each of its offerings with a short audio signal, which helps the user prepare the pipette dispenser in time, insert a sample, and begin the analysis. The dispenser automatically takes the required amount of sample and then distributes it to the slide in sequence of 10 μl. As the sample passes through the slide layers, biochemical reactions occur that result in successive color changes. The VetTest analyzer optical system determines colors and their intensity. The analyzer converts the measured results into numerical measure values that are displayed on the analyzer screen and printed.

Then, phagocytic activity of leukocytes was determined using the daily agar culture of Staphylococcus aureus, lysozyme activity of blood plasma – by agar culture Micrococcus lysodeikticus, bactericidal activity of blood serum – using daily agar culture of Escherichia coli, as well as the amount of antibodies in the serum was measured on a photoelectrocolorimeter FEK-56M (Zagorsky Optical and Mechanical Plant, Russia).

Specific antibodies (immunity tensions) against Gamborough disease (IBB) in the response of immunoenzyme assay (IFA), Newcastle disease and egg reduction syndrome (EDS-76) in the hemagglutination delay reaction (RTGA) with specific antigens at the Federal Centre for Animal
Health (Russia, Vladimir) were determined in blood serum.

Live weight, average daily growth of poultry was determined by weighing data; Poultry productivity per carrying chicken - according to egg production data for the observed period; The incidence and safety of poultry according to veterinary reports.

Veterinary and sanitary examination of poultry meat was carried out in accordance with interstate standards GOST RF 21784-76, GOST RF 7269-2015 (ISO 3166) 004-97, GOST RF 7702.2.0-2016 (ISO 3166) 004-97 [25-27].

Experimental data were processed by the method of variation statistics on the reliability of the difference between the compared indicators (P <0.05-0.001) using the Microsoft Office Excel software package.

3. Research results

It was established that the live weight did not have significant differences in the chicks of the experimental and control groups at day-old. However, on the 30th day, the chickens of the experimental groups outnumbered the poultry of the control group. Thus, the live weight of the chickens of the 1st experimental group was higher by 1.5%, the 2nd - 2.5% and the 3rd - by 4.5% (P<0.01). Subsequently, the chickens of the experimental groups grew and developed better than the young of the control group. On the 110th day, the live weight of young poultry of the 1st, 2nd and 3rd experimental groups exceeded, by comparison with the control, 5.4%, 7.1% and 10.1% (P <0.01-0.001) respectively.

Analyzing the average daily gains in live weight of young birds, it should be noted that they were similar to the dynamics of weight gain (table 1).

| Age of bird, days | Control group | 1 Experimental group of | 2 Experimental group of | 3 Experimental group of |
|------------------|---------------|-------------------------|-------------------------|-------------------------|
| 10               | 4.0±0.3       | 3.9±0.25                | 3.9±0.27                | 4.0±0.6                 |
| 30               | 6.3±0.4       | 7.6±0.21**              | 7.7±0.35**              | 8.0±0.25***             |
| 60               | 11.8±0.5      | 12.8±0.6                | 13.2±0.4*               | 13.6±0.5**              |
| 90               | 14.0±0.3      | 15.1±0.5                | 15.2±0.6                | 15.7±0.4**              |
| 110              | 13.1±1.1      | 13.7±0.9                | 13.9±0.6                | 14.4±0.7                |

Notes: * P<0.05, ** P<0.01, *** P<0.001.

The dynamics of changes in the absolute and average daily gain in live weight of young birds of the experimental groups depended on the dose of the drug. Maximum efficiency is established when using this immunostimulant in a dose of 0.15 ml per 1 kg of body weight.

The hematological profile of birds is presented in table 2.

It was established that the administration of the PV-1 immunostimulator contributed to an increase in the number of erythrocytes in the blood of birds of the 1st experimental group by 9.5-15.4% (P>0.05), the 2nd - by 13.8-18.1% (P>0.05) and the 3rd experimental one - by 16.3-22.1% (P<0.05-0.01) as compared with the control. The hemoglobin content in the blood of experimental groups was also higher than in the control: in the 1st experimental - by 2.0-13.1%, in the 2nd one - by 5.5-15.7% and in the 3rd experimental one - by 7.9-20.1% (P<0.05). The number of leukocytes in birds increased with age, which is apparently due to the formation of the functional activity of the blood-forming organs and the immune system. In birds of the experimental groups, the content of leukocytes at 30, 60 and 110 days of age was higher than in the control: in the 1st experimental one - by 4.5-15.3% (P<0.05), in the 2nd - by 3.8-18.1% (P<0.05) and in the 3rd experimental one - by 3.0% (P<0.05) - 20.7% (P<0.01). The increase in the number of erythrocytes and the concentration of hemoglobin in the blood of birds in the experimental groups against the background of immune correction indicates an improvement in their hematopoiesis, and an increase in the number of leukocytes indicates an activation of the cellular protective factors of the body.
The formation of protein metabolism in young animals to the level of adult birds, and further the stabilization of this exchange occurs. It should be noted that the content of total protein in birds of the 1st experimental group was higher than in the control by 3.4-4.7% (P<0.05), the 2nd - by 4.4-6.7% (P<0.05) and 3rd experimental - by 5.4-11.2% (P<0.05-0.01). The total protein content in the experimental groups increased due to an increase in the number of albumin and gamma globulins.

The study of immunological blood parameters (table 4) showed that the phagocytic activity of leukocytes increases with the age of the bird. This indicator was higher in young experimental groups.
in the 60-day age compared to the control, in the 1st experimental group by 9.8%, in the 2nd - by 18.2% and in the 3rd - by 20.4% (P < 0.05-0.01), at 90-day age - by 2.6%, 6.8 and 7.2% (P < 0.05), at 110-day age - at 14, 6%, 20.5%, 29.5% (P < 0.05-0.001), respectively. The absorption capacity of pseudo-eosinophils varied. In the 2nd and 3rd experimental groups, the phagocytic index was higher by 1.7-11.7% (P < 0.05-0.01) than in the control.

Table 4. Dynamics of immunological indicators of poultry.

| Group of birds | Age, day | Phagocytic activity, % | Phagocytic index | Lysozyme activity, % | Bactericidal activity, % |
|---------------|----------|------------------------|-----------------|----------------------|-------------------------|
| Control       | 60       | 22.5±1.2               | 1.62±0.08       | 25.3±1.0             | 49.8±1.1                |
|               | 90       | 26.5±0.9               | 1.78±0.09       | 38.9±1.2             | 52.3±1.2                |
|               | 110      | 34.2±1.6               | 1.79±0.05       | 42.9±1.0             | 54.7±1.1                |
| 1 Experimental| 60       | 24.7±1.0               | 1.72±0.06       | 32.8±2.2**           | 55.6±1.2**              |
|               | 90       | 27.2±1.1               | 1.81±0.04       | 45.0±1.6**           | 56.3±1.1*               |
|               | 110      | 39.2±1.3*              | 1.80±0.08       | 48.8±1.3**           | 58.0±0.9*               |
| 2 Experimental| 60       | 26.6±1.3*              | 1.78±0.1*       | 36.9±3.2**           | 57.7±1.85**             |
|               | 90       | 39.2±1.3*              | 1.86±0.12       | 51.4±0.5**           | 58.1±0.8**              |
|               | 110      | 41.2±1.9**             | 1.82±0.09       | 53.1±2.3**           | 60.7±0.6**              |
| 3 Experimental| 60       | 28.4±0.9*              | 1.82±0.1*       | 54.1±2.9**           | 58.8±1.1**              |
|               | 110      | 44.3±1.3***            | 1.86±0.11*      | 57.9±2.4**           | 61.6±1.0**              |

Notes: *P<0.05, **P<0.01, ***P<0.001.

The lysozyme activity of the blood serum of birds in the 1st, 2nd and 3rd experimental groups throughout the entire study period was higher than in the control: at 60 days age - by 29.6%, 45.8 and 47.0%, in 90 days - by 15.7%, 32.1 and 39.0%, in 110 days - by 13.7%, 23.8 and 35% (P <0.01-0.001), respectively.

The bactericidal activity of the serum of birds of the 1st, 2nd and 3rd experimental was also higher than in the control: at 60 days age - by 11.6%, 15.9 and 15.8%, in the 90 days age - by 7.6%, 11.1 and 12.4%, in the 110-day age - by 6.0%, 11.0 and 12.6% (P <0.05-0.01), respectively.

Thus, the administration of the PV-1 immunostimulant in growing chickens contributed to the improvement of erythropoiesis, leukopoiesis and protein metabolism, as well as an increase in the immunological parameters of the body of the young.

We have studied the effect of the PV-1 immunostimulant on the peculiarities of the formation of immunity in the vaccination of birds against the diseases of Gumboro, Newcastle, and EDS-76.

Immunization of chickens against Gumboro disease led to a maximum accumulation of specific antibody titers 30 days after vaccination (table 5).

Table 5. Accumulation of antibody titer against Gumboro disease.

| Group of birds | The average antibody titre (in EIA units), after days |
|---------------|------------------------------------------------------|
|               | 30          | 60          | 90          |
| Indicator     | % over control | % over control | % over control |
| Control       | 6306        | 100         | 3257        | 100         | 2257        | 100         |
| 1 Experimental| 6420        | 101.8       | 3171        | 97.3        | 3040        | 134.7       |
| 2 Experimental| 7205        | 114.2       | 4919        | 151.0       | 4822        | 212.7       |
| 3 Experimental| 9671        | 153.3       | 5395        | 165.6       | 5211        | 229.9       |

In the experimental groups, antibody titers were higher than in the control: in the 1st experimental - by 1.8%, in the 2nd one - by 14.2% and in the 3rd one - by 53.3%. In subsequent periods of research, the titer of specific antibodies decreased in all experimental groups: on the 60th day after vaccination
by 31.7-50.6%, on the 120th day - by 33.1-64.2%. It should be noted that the intensity of immunity against Gamboro disease against the background of the use of PV-1 immunostimulant in all experimental groups was maintained for 120 days at the level of 47.4-66.9%, and in the control antibody titers decreased in 2.8 times.

The maximum accumulation of specific neutralizing antibodies against Newcastle's disease was noted 60 days after the vaccination of the bird (table 6).

Table 6. Dynamics of antibody titer against Newcastle disease.

| Age after vaccination | Group of birds | Control | 1 Experimental | 2 Experimental | 3 Experimental |
|-----------------------|----------------|---------|----------------|---------------|---------------|
| 30                    | 4.5/100        | 5.0/111.1 | 6.0/133.3 | 6.75/150.0 |
| 60                    | 5.4/100        | 7.1/131.5 | 7.5/138.9 | 8.1/150.0 |
| 90                    | 5.0/100        | 6.0/120.0 | 6.5/130.0 | 7.4/148.0 |
| 120                   | 4.6/100        | 5.8/126.1 | 6.4/139.1 | 6.7/145.6 |
| 160                   | 3.4/100        | 5.0/147.0 | 5.5/161.8 | 5.4/158.8 |
| 190                   | 3.2/100        | 4.0/125.0 | 4.5/140.6 | 4.7/146.9 |
| 250                   | 3.2/100        | 3.3/103.1 | 3.9/121.9 | 4.3/134.4 |
| 360                   | 4.7/100        | 4.8/102.1 | 5.8/123.4 | 5.6/119.1 |

Notes: In the numerator - the average antibody titer in Ig2; in the denominator - the percentage compared to control.

In the 1st experimental group, the antibody titers were higher compared to the control by 31.5%, in the 2nd - by 38.9%, in the 3rd experimental - by 50.0%. The level of antibodies gradually decreased in all experimental groups by the 90th day. But in the experimental groups, antibody titers were higher than in the control: in the 1st experimental - by 20.0%, in the 2nd one - by 30.0%, in the 3rd one - by 48.0%. The administration of the PV-1 immunostimulant contributed to maintaining the titer of neutralizing antibodies at a high level up to 160 days after immunization, whereas in the control group it significantly decreased from the 90th day after vaccination.

Immunization of young poultry against the disease of EDS-76 against the background of the administration of the PV-1 immunostimulant led to an increase in the antibody titer in the 1st experimental group by 17.8%, in the 2nd one - by 24.3%, in the 3rd experimental group - by 26.5% compared with the control group. On the 100th, 170th, and 270th days after vaccination, the titers of neutralizing antibodies in the control group of birds gradually decreased. But in the experimental groups, it was high, especially when using PV-1 at a dose of 0.15 ml per 1 kg of live weight of the bird.

Thus, the administration of the PV-1 immunostimulant contributes to an increase in the immunity tension during the vaccination of birds against Gamboro diseases, Newcastle and EDS-76. At the same time, the duration of preservation of specific virus neutralizing antibodies was increased compared with the control.

It was established that if the egg production for the initial hen for 68 weeks of life in the control group was 196, then in the 1st experimental group it was 9.2% more, in the 2nd experimental one - by 9.7%, in the 3rd experimental - 17.8%.

Based on the average hen, the egg production in the control group was 220 pieces. In the experimental groups, it was higher than in the control: in the 1st experimental one - by 4.1%, in the 2nd one - by 5.4% and in the 3rd one - by 11.8%. The laying hens of the control group reached 50% egg-laying at the age of 158 days, the 1st and 2nd experimental groups - 152 days, the 3rd experimental - 155 days. The peak of egg production of laying hens of experimental groups reached the 6th month of egg-laying: the control group - at the 199-day age, the 1st experimental group - at the 192-day, 2nd experimental - the 200-day, the 3rd experimental - at the 183-day age.

One of the important indicators in the practice of industrial poultry is considered the mass of eggs.
The mass of eggs at the beginning of the productive period was low and amounted to 52.3 ± 0.2 g in birds of the control group. In the 1st experimental group it was higher by 0.5%, in the 2nd by 1.3% (P <0.05), in the 3rd - by 4.6% (P <0.01) than in control. At the height of the egg-laying, the eggs mass was higher and amounted to 56.8 ± 0.13 g in the control group, 57.1 ± 0.13 g in the 1st test group, 57.8±0.2 g in the 2nd one (Р <0.05), in the 3rd experimental one - 58.1 ± 0.21 g (Р <0.01). It was revealed that eggs of young chickens contain more protein (58.5-59.6%) and less yolk (26.3-27.1%) than in older chickens (57.6-58.1% and 28.3-28.5%, respectively). The administration of PV-1 had an effect on the egg protein mass in the initial period of egg production, i.e. it increased (P <0.01). The mass of yolk in the context of the experimental groups of birds did not differ significant changes.

The safety of the birds for 4 weeks of cultivation was 98.3% in the control group, 98.3% in the 1st experimental group, and 100% in the 2nd and 3rd experimental groups. The safety of the birds from the 5th to the 16th week in the control group was 86.6%, in the 1st experimental group – 96.6%, in the 2nd and 3rd experimental groups – 99.8%. Apparently, this is due to the fact that the drug PV-1 contributes to an increase in the nonspecific resistance of the organism of the bird and its resistance to the action of adverse environmental factors.

Studying the meat productivity of poultry grown using the PV-1 immunostimulant, an increase in slaughter yield of 1.7–5.0% was established, the yield of edible parts increased in the experimental groups from 62 ± 3.2 to 155 ± 3.4 g (P < 0.001).

4. Conclusion
We recommend using the PV-1 immunostimulator in a dose of 0.15 ml per 1 kg of live weight by feeding with feed once a day for 10 days with a 10-day break to increase the productivity, non-specific resistance, and preservation of the birds.

In order to increase the immunity tension when vaccinating birds against Gumboro diseases, Newcastle and EDS-76, we recommend using the PV-1 immunostimulatant in a dose of 0.1-0.15 ml per 1 kg of body weight by feeding with feed 10-12 days before immunization.

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