Dynamics of passive immunity formation against the causative agent of infectious bursal disease and its immunological significance

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Abstract. In the study, the spread and pathogenetic features of Newcastle disease under industrial poultry farming conditions with high stocking density were analyzed. Investigated the impact of Newcastle disease virus on the respiratory, digestive, and central nervous systems of poultry. Revealed the role of maternally derived passive immunity in forming the initial level of protection in newly hatched chicks during the early postnatal period. Studied the dynamics of maternal antibody levels and the duration of their persistence in the organism of young birds at different developmental stages. Determined the influence of natal and postnatal developmental periods on the formation of the immune system and the functional activity of lymphoid organs. Established the dependence of the immune response to vaccination on the initial immune status and physiological condition of poultry. Formed an understanding of changes in immunological indicators during the growth and development of chicks under industrial conditions. Proposed the consideration of immunological monitoring results when designing preventive measures against the disease. Developed a rationale for optimizing vaccination schemes based on age-related and immunological characteristics of poultry. Substantiated the application of an integrated immunoprophylaxis approach aimed at increasing resistance to infection and reducing disease spread.

1 Relevance of the Topic

Achieving high performance in the poultry industry worldwide largely depends on the effective immunoprophylaxis of infectious diseases. It is well recognized that in all industrialized poultry farms, the effective immunoprophylaxis of pantropic viral infections (avian influenza, Newcastle disease, and infectious bronchitis) constitutes a primary task within preventive health programs. According to the World Organisation for Animal Health (WOAH), the continued reporting of highly pathogenic avian influenza and Newcastle disease among poultry in certain countries is a matter of serious concern. These diseases

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can spread transboundary through the migration of wild birds. Therefore, the effective immunoprophylaxis of infectious diseases remains an urgent priority.

In our country, industrial poultry farming has been developing since the 1960s. Throughout this period, the effective immunoprophylaxis of Newcastle disease has consistently been regarded as one of the most important preventive measures. The disease has been detected several times, and quarantine measures have been implemented to control outbreaks. Consequently, the effective immunoprophylaxis of pantropic viral infections (avian influenza, Newcastle disease, and infectious bronchitis) remains a significant responsibility for specialists today.

It should be emphasized that Newcastle disease in poultry belongs to the group of pantropic viral infections. A pantropic infection is characterized by the ability of the pathogen to replicate in various tissues and cell types of the macroorganism. For this reason, the immune system encounters considerable difficulty in combating such infections. The continuous development of new vaccines against pantropic infections and the ongoing scientific and practical research efforts reflect the importance of this issue. Undoubtedly, immunological indicators during the natal and postnatal development of chicks are of particular importance.

In all industrial poultry farms, routine vaccination against Newcastle disease is implemented, and the maintenance of post-vaccinal immunity levels above 80% is required. This also applies to breeder populations; therefore, hatching eggs are expected to contain specific antibodies [2].

From a broader, phylogenetic perspective, it is logical to recognize that in all amniotes, passive immunity serves as a beneficial evolutionary mechanism that protects offspring from pathogenic microbial exposure during the early stages of postnatal development. However, scientific studies on Newcastle disease immunoprophylaxis have accumulated contradictory data regarding the influence of passive immunity on vaccination outcomes [1].

Indeed, according to some researchers, regular vaccination of breeder hens against Newcastle disease may lead to excessively high levels of passive immunity in chicks and consequently weaken post-vaccinal immunity, resulting in an interference phenomenon. In contrast, other sources [5,7] report that the timing of breeder hen vaccination prior to egg collection does not significantly affect the duration of passive immunity in chicks. For example, when hens were vaccinated three months before egg collection, passive antibodies were detected in chicks' sera up to 15 days of age; when vaccinated 20 days before egg collection, antibodies were detected up to 20 days of age. Accordingly, current guidelines recommend vaccinating chicks against Newcastle disease starting from 15–20 days of age.

According to F.A. Niyazov, although passive immunity is high in day-old chicks, it declines to zero by day 12 and does not interfere with the formation of post-vaccinal immunity. At the same time, it has been noted that maternally derived antibodies transmitted through the egg may suppress post-vaccinal immunity. Some authors report that passive antibodies may even inhibit immunogenesis in response to a virulent virus entering the chick's body, thereby causing an interference phenomenon [8,9].

Comparative experimental studies involving chicks with high and low titers of passive immunity [6,11] indicate that high titers of passive immunity negatively affect post-vaccinal immunity in 14-day-old chicks. Conversely, alternative findings demonstrate that even in day-old chicks with high titers of passive immunity, maternal antibodies do not prevent the development of post-vaccinal immunity following vaccination against Newcastle disease [8].

Moreover, other authors report that not only maternal antibodies but also immune serum administered to chicks against Newcastle disease may positively influence post-vaccinal immunity [12,13].

Another complex aspect of the issue is that when chicks are vaccinated intramuscularly and intranasally against Newcastle disease, high levels of passive immunity negatively affect post-vaccinal immunity in the case of intramuscular administration, but do not interfere with the effectiveness of intranasal vaccination [3].

Even more unusual findings indicate that the titer of passive antibodies against Newcastle disease may increase during days 3–7 of postnatal development. This is attributed to the continued absorption of immunoglobulins from the yolk sac into the bloodstream for approximately one week after hatching [4,10].

Despite the contradictory and sometimes complex data regarding the relationship between passive and active immunity described above, it can be concluded that scheduling both the initial vaccination after hatching and subsequent vaccinations at 10–12 day intervals ensures an optimal interaction between antibodies and vaccine antigens.

However, it would be premature to consider this interval fully optimal. Factors such as anti-idiotypic antibodies, their immunogenic properties, and the phenomenon of immunological resonance remain insufficiently elucidated. Therefore, the interval between vaccination and revaccination may still be regarded as suboptimal.

2 Research Methods

The study employed refractometric and electrophoretic methods, as well as immunological solution-based techniques. The levels of IgM and IgG were determined using Mancini's radial immunodiffusion method. Immunoserological analysis was performed using the hemagglutination inhibition (HI) test.

3 Research Results

Positive outcomes obtained in epizootological and experimental studies demonstrated that passive immunity, beginning from 15 days of age, participates in the formation of active immunity in chicks by initiating the synthesis of anti-idiotypic antibodies. These findings necessitated a detailed study of the ontogenesis of maternally derived immunity.

To achieve this, the timing of the appearance and persistence of immunoglobulins from the incubation egg in the bloodstream of developing chicken embryos was first determined.

The investigations were carried out starting from 13–14-day-old embryos. In total, 500 embryos were used in the experiment. Beginning from day 13–14 of incubation, blood samples were collected from 10 embryos every 6 hours. The experiment continued for one week after hatching.

After separating the serum from the blood obtained from embryos or chicks, the total protein content and its fractions, as well as the levels of IgM and IgG, were determined. In addition, the antibody titers against the causative agent of Newcastle disease in the blood serum were measured. The experimental results are presented in Tables 1–2–3–4–5.

The indicators in Table 1 show that the immunobiological parameters of blood serum in 13–14-day-old chicks exhibited some degree of daily (circadian) variability.

The total protein content increased during the day from 3.45 ± 0.25 g% at 6:00 AM to 3.70 ± 0.505 g% at 12:00 AM, with an average value of 3.535 g%. No statistically significant difference was observed ($P>0.05$), indicating that protein metabolism processes are stabilizing at this age.

Albumin levels varied from 1.19 ± 0.10 g% to 1.36 ± 0.15 g%, with an average of 1.30 g%. This parameter showed a statistically significant difference ($P<0.05$), suggesting active synthesis of the albumin fraction.

Table 1. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 13–14 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	3.45±0.25	3.52±0.30	3.47±0.405	3.70±0.505	3.535*
Albumins, g%	1.19±0.10	1.35±0.14	1.33±0.12	1.36±0.15	1.30**
α-globulins, g%	1.21±0.12	1.59±0.10	1.14±0.14	1.19±0.13	1.282
β-globulins, g%	1.04±0.14	0.98±0.12	1.02±0.11	1.12±0.15	1.04
γ-globulins, g%	-	-	-	-	
IgM, mg/ml	-	-	-	-	
IgG, mg/ml	-	-	-	-	
Immune potency, %	-	-	-	-	

Note: * – level of reliability (* P>0.05, ** P<0.05).

α-Globulins ranged from 1.14 g% to 1.59 g%, with an average of 1.282 g%. The maximum value (1.59 ± 0.10 g%) was recorded at 12:00 PM.

β-Globulin levels remained relatively stable, ranging from 0.98 g% to 1.12 g%, with an average of 1.04 g%.

γ-Globulins, IgM, IgG, and immune potency were not detected (—), indicating that humoral immunity was not yet sufficiently developed at this age or could not be measured within the scope of the study.

Overall, during the 13–14-day period, protein fractions demonstrated daily biorhythmic variations, with a particularly reliable upward trend observed in the albumin fraction.

Table 2. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 14–15 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	24	24	24	24	Avarage
Total protein, g%	3.70±0.52	3.72±0.45	3.54±0.40	3.87±0.53	3.707*
Albumins, g%	1.43±0.12	1.43±0.14	1.55±0.15	1.49±0.21	1.475**
α-globulins, g%	1.19±0.10	1.18±0.15	1.22±0.13	1.22±0.17	1.202
β-globulins, g%	1.08±0.12	1.10±0.13	1.02±0.15	1.05±0.15	1.06
γ-globulins, g%	-	-	-	-	
IgM, mg/ml	-	-	-	-	
IgG, mg/ml	-	-	-	-	
Immune potency, %	-	-	-	-	

Note: * – level of reliability (* P>0.05, ** P<0.05).

The indicators in Table 2 show that the immunobiological parameters of blood serum in 14–15-day-old chicks were slightly higher compared to the 13–14-day period.

The total protein content ranged from 3.54 ± 0.40 g% to 3.87 ± 0.53 g%, with an average of 3.707 g% (P>0.05). The highest value was recorded at 12:00 AM.

Albumin levels varied from 1.43 ± 0.12 g% to 1.55 ± 0.15 g%, with an average of 1.475 g%, showing a statistically significant difference (P<0.05). This indicates increased activity of protein synthesis at this stage of development.

α-Globulins remained relatively stable within the range of 1.18–1.22 g%, averaging 1.202 g%. β-Globulins ranged from 1.02–1.10 g%, with an average of 1.06 g%. γ-Globulins, IgM, IgG, and immune potency were still undetected at this stage.

In summary, the increase in total protein and, in particular, the albumin fraction during the 14–15-day period reflects the intensification of metabolic and immunobiological

processes. At this stage, using the applied methods, the quantity of γ-globulins in embryonic blood serum was not detected, indicating that they were either very low or had not yet entered the bloodstream (Table 3).

By 15–16 days of age, the immunobiological parameters of blood serum were observed to be significantly more active compared to earlier periods. At this stage, not only the protein fractions but also γ-globulins were detected, indicating the initiation of humoral immunity formation.

Total protein increased from 3.44 ± 0.425 g% at 6:00 AM to 4.29 ± 0.565 g% at 12:00 AM, with an average of 3.955 g%. The marked increase during evening and night hours reflects heightened metabolic activity.

Albumins ranged from 1.33–1.68 g%, with an average of 1.470 g%, and the highest value was recorded at 6:00 AM, indicating active protein synthesis by the liver.

α-Globulins ranged from 1.08–1.25 g%, averaging 1.177 g%, and remained relatively stable. β-Globulins varied from 1.11–1.42 g%, with an average of 1.235 g%, showing higher values in the morning (6:00 AM).

Importantly, γ-globulins were detected for the first time at this stage (0.18–0.50 g%), with an average of 0.345 g%. The highest value was recorded at 12:00 AM (0.50 ± 0.049 g%), indicating the initiation of immunoglobulin synthesis.

IgM and IgG were not yet detected, but the appearance of γ-globulins suggests the early formation of humoral immune components. Immune potency reached 44% at 12:00 AM, reflecting increasing immunoreactivity in the young organism.

Overall, during the 15–16-day period, protein metabolism and immunobiological processes became significantly more active. The appearance of the γ-globulin fraction and the recorded immune potency indicate that chicks have entered the stage of humoral immune system development. The higher values observed during night hours in daily dynamics may be associated with biological rhythms (Table 3).

Table 3. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 15–16 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	3.44±0.425	4.01±0.520	4.08±0.480	4.29±0.565	3.955
Albumins, g%	1.68±0.160	1.33±0.152	1.39±0.200	1.48±0.202	1.470
α-globulins, g%	1.08±0.130	1.19±0.145	1.25±0.112	1.19±0.168	1.177
β-globulins, g%	1.42±0.138	1.16±0.125	1.25±0.130	1.11±0.176	1.235
γ-globulins, g%	0.18±0.02	0.44±0.012	0.26±0.022	0.50±0.049	0.345
IgM, mg/ml	-	-	-	-	
IgG, mg/ml	-	-	-	-	
Immune potency, %	-	-	-	44	

The increase in total protein content, as well as the appearance of γ-globulins in embryonic blood, was first observed starting from 15–16 days of development. IgM and IgG levels remained extremely low and were often detectable only as faint traces in immunodiffusion assays, beginning from 16–17 days of age (Table 4).

The indicators in Table 4 show that the immunobiological parameters of blood serum in 16–17-day-old chicks were significantly higher compared to earlier developmental stages. This period corresponds to the active formation phase of humoral immunity.

Total protein content ranged from 4.62 ± 0.42 g% at 6:00 AM to 4.92 ± 0.38 g% at 6:00 PM, with an average of 4.77 g%. The consistently high values indicate accelerated protein metabolism and synthetic activity.

Albumin levels varied from 2.16–2.25 g%, averaging 2.212 g%, and showed statistically high reliability (P<0.01), reflecting increased liver functional activity and intensified anabolic processes.

α-Globulins remained stable between 1.21–1.26 g%, averaging 1.242 g%. β-Globulins ranged from 0.41–0.71 g%, averaging 0.56 g%, with relatively higher values observed at 12:00 PM and 6:00 PM.

Table 4. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 16–17 Days of Natal (Embryonic) Development, M±m (n=10)

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	4.62±0.42	4.83±0.56	4.92±0.38	4.71±0.68	4.77
Albumins, g%	2.22±0.180	2.25±0.204	2.22±0.158	2.16±0.316	2.212*
α-globulins, g%	1.21±0.200	1.25±0.133	1.25±0.128	1.26±0.1775	1.242
β-globulins, g%	0.42±0.04	0.71±0.024	0.70±0.044	0.41±0.08	0.56
γ-globulins, g%	0.51±0.065	0.64±0.073	0.76±0.020	0.86±0.099	0.69
IgM, mg/ml	Izlari	0.13±0.01	-	0.23±0.01	0.18
IgG, mg/ml	1.40±0.150	1.15±0.120	1.55±0.184	1.59±0.203	1.422
Immune potency, %	36	68	56	64	

Note: * – level of reliability (* P<0.01).

γ-Globulin levels increased markedly (0.51–0.86 g%), averaging 0.69 g%, with the highest value recorded at 12:00 AM (0.86 ± 0.099 g%). This demonstrates intensified immunoglobulin synthesis and the active formation of humoral immunity.

IgM was detected in trace amounts at 6:00 AM and 6:00 PM, 0.13 ± 0.01 mg/ml at 12:00 PM, and 0.23 ± 0.01 mg/ml at 12:00 AM, averaging 0.18 mg/ml. This indicates activation of primary immune response mechanisms.

IgG levels ranged from 1.15–1.59 mg/ml, averaging 1.422 mg/ml, with the maximum value at 12:00 AM, reflecting the establishment of stable humoral immunity.

Immune potency varied between 36–68%, with the highest level at 12:00 PM (68%), indicating a significant increase in immunoreactivity at this stage of development.

During the 16–17-day natal development period, chicks exhibited increased protein metabolism, particularly in the albumin fraction (P<0.01), as well as rising levels of γ-globulins and immunoglobulins (IgM, IgG). This period represents the active phase of humoral immunity formation, characterized by higher immunological indicators during evening and night hours due to circadian rhythms.

Importantly, antibodies against the Newcastle disease virus were detected in blood serum as soon as traces of immunoglobulins appeared on immunodiffusion plates, indicating the initiation of specific immune defense (Table 4).

Based on the indicators in Table 5, in 17–18-day-old embryos, immunoglobulins were consistently detected in the blood and gradually increased (P<0.05).

At this stage, the immunobiological parameters of blood serum were highly stabilized, reflecting the active formation and strengthening of humoral immunity.

Total protein ranged from 4.76–4.87 g%, with an average of 4.835 g%. Values remained nearly stable throughout the day, indicating balanced protein metabolism at this stage.

Albumin levels ranged from 2.22–2.28 g%, averaging 2.255 g%. The consistently high albumin fraction indicates a well-developed liver synthetic function.

Table 5. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 17–18 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	4.87±0.56	4.76±0.44	4.84±0.52	4.87±0.69	4.835
Albumins, g%	2.22±0.24	2.24±0.18	2.28±0.16	2.28±0.32	2.255
α-globulins, g%	1.83±0.126	0.70±0.20	0.83±0.132	0.72±0.148	1.02
β-globulins, g%	0.78±0.088	0.82±0.102	0.76±0.098	0.77±0.112	0.782
γ-globulins, g%	0.91±0.096	0.91±0.064	0.99±0.076	1.09±0.139	0.975
IgM, mg/ml	0.17±0.012	0.40±0.024	0.55±0.036	0.54±0.0593	0.415*
IgG, mg/ml	1.34±0.120	1.49±0166.	1.44±0.180	1.46±0.204	1.432*
Immune potency, %	82	100	92	100	

Note: * – level of reliability (* P<0.05).

α-Globulins varied from 0.70–1.83 g%, averaging 1.02 g%. A higher value was observed at 6:00 AM (1.83 ± 0.126 g%), likely associated with circadian rhythm.

β-Globulins remained relatively stable, ranging from 0.76–0.82 g%, with an average of 0.782 g%.

γ-Globulin levels ranged from 0.91–1.09 g%, averaging 0.975 g%, with the highest value recorded at 12:00 AM (1.09 ± 0.139 g%), reflecting intensified immunoglobulin synthesis.

IgM increased from 0.17 ± 0.012 mg/ml to 0.55 ± 0.036 mg/ml, averaging 0.415 mg/ml, with a statistically significant difference (*P<0.05). Higher values were noted at 6:00 PM and 12:00 AM, indicating enhanced primary immune response.

IgG ranged from 1.34–1.49 mg/ml, averaging 1.432 mg/ml (*P<0.05), reflecting the formation of a stable humoral immune response.

Immune potency varied between 82–100%, reaching a maximum of 100% at 12:00 PM and 12:00 AM, indicating the highest level of immunoreactivity at this stage.

During the 17–18-day natal development period, chicks showed an increase in protein fractions, especially γ-globulins and immunoglobulins (IgM, IgG). The achievement of 100% immune potency indicates the completion of humoral immunity formation. This stage is characterized by functional maturation of the immune system and a significant increase in the organism's resistance to external factors (Table 6).

Table 6. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 18–19 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	4.68±0.422	4.81±0.348	4.91±0.532	4.87±0.688	4.81
Albumins, g%	2.09±0.18	2.15±0.22	2.29±0.26	2.19±0.311	2.18
α-globulins, g%	0.79±0.098	1.84±0.116	0.85±0.125	0.70±0.149	1.045
β-globulins, g%	0.61±0.042	0.62±0.066	0.57±0.060	0.69±0.089	0.622
γ-globulins, g%	1.19±0.112	1.18±0.148	1.28±0.136	1.34±0.178	1.247
IgM, mg/ml	0.26±0.012	0.32±0.038	0.33±0.024	0.52±0.051	0.357
IgG, mg/ml	1.61±0.15	1.36±0.12	1.70±0.14	1.98±0.202	1.662
Immune potency, %	76	100	100	80	

At this stage, the sharp increase in γ-globulins, particularly immunoglobulins (P<0.01), was not reflected in the total protein content. This indicates that albumins were being actively utilized by the organism.

The levels of α- and β-globulins initially increased synchronously with total protein during the early days of the study (Tables 3–4), but from days 17–18 onward, they gradually decreased in a statistically significant manner, eventually stabilizing toward the end of the experiment (Tables 5–6).

The appearance of immunoglobulins in the blood from 16–17 and 17–18 days of natal development is of immunological significance. In particular, even the low concentration of IgM immunoglobulins was found to be functionally important (Tables 5–9).

Table 7. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 19–20 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	4.78±0.422	4.79±0.584	5.03±0.604	5.08±0.703	4.92*
Albumins, g%	2.15±0.266	2.03±0.188	2.15±0.184	2.15±0.303	2.12
α-globulins, g%	0.72±0.098	1.69±0.084	0.65±0.105	0.55±0.129	0.902
β-globulins, g%	0.58±0.052	0.51±0.024	0.63±0.036	0.54±0.080	0.565
γ-globulins, g%	1.35±0.112	1.46±0.106	1.62±0.104	1.86±0.22	1.572
IgM, mg/ml	0.57±0.09	0.71±0.088	0.65±0.076	0.96±0.103	0.722**
IgG, mg/ml	1.70±0.175	1.48±0.163	1.88±0.128	1.96±0.250	1.755**
Immune potency, %	88	100	100	96	

Note: * – level of reliability (*P<0.01, **P<0.01).

Based on Table 7, in 19–20-day-old embryos, the immunobiological parameters of blood serum were maximally activated. This period reflects the functional maturation of the immune system.

Total protein increased from 4.78 ± 0.422 g% to 5.08 ± 0.703 g%, with an average of 4.92 g%, showing statistically significant reliability (*P<0.01). The highest values were recorded at 6:00 PM and 12:00 AM, indicating intensified protein synthesis and metabolic activity.

Albumin ranged from 2.03–2.15 g%, averaging 2.12 g%, remaining relatively stable, reflecting normal liver synthetic function.

α-Globulins varied between 0.55–1.69 g%, with an average of 0.902 g%. The peak at 12:00 PM (1.69 ± 0.084 g%) may relate to circadian rhythms.

β-Globulins ranged from 0.51–0.63 g%, averaging 0.565 g%, and remained relatively stable.

γ-Globulins significantly increased to 1.35–1.86 g%, averaging 1.572 g%, with the highest value at 12:00 AM (1.86 ± 0.22 g%), indicating high immunoglobulin synthesis activity.

IgM ranged from 0.57–0.96 mg/ml, averaging 0.722 mg/ml (**P<0.01), with the highest value at 12:00 AM, showing an intensified primary immune response.

IgG ranged from 1.48–1.96 mg/ml, averaging 1.755 mg/ml (**P<0.01), with peak values at 6:00 PM and 12:00 AM, reflecting a stable and fully formed humoral immune response.

Immune potency varied between 88–100%, reaching 100% at 12:00 PM and 6:00 PM, indicating that the organism's immunoreactivity had reached a high level.

During 19–20 days of natal development, chicks exhibited significant increases in total protein, γ-globulins, IgM, and IgG (P<0.01). The near-maximal immune potency indicates the full formation of humoral immunity and a high level of resistance to external

environmental factors. This period represents the functional maturity and biological stabilization of the immune system.

Notably, both total protein and γ-globulins, particularly immunoglobulins, sharply increased in embryos approaching hatching (19–20 days) (Table 7, P<0.01). Another key observation is that the difference in immunoglobulin levels between newly hatched chicks and the final days of embryonic development was significant only for IgG, with hatched chicks showing 2–3 times higher levels (P>0.01). Finally, during the last three days of embryonic development, the passive immune potency, as indicated by antibody titers, remained consistently high, as detailed in Tables 7–8.

Table 8. Dynamics of Immunobiological Parameters of Blood Serum in Chickens During 20–21 Days of Natal (Embryonic) Development, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	4.62±0.456	4.76±0.746	4.81±0.566	4.85±0.673	4.76
Albumins, g%	1.72±0.180	1.90±0.164	1.95±0.192	1.59±0.25	1.79
α-globulins, g%	0.76±0.040	0.52±0.054	0.40±0.022	0.82±0.089	0.632
β-globulins, g%	0.62±0.080	0.74±0.068	0.84±0.092	0.88±0.103	0.77
γ-globulins, g%	1.52±0.120	1.59±0.134	1.32±0.104	1.38±0.20	1.452
IgM, mg/ml	0.88±0.098	1.12±0.106	1.28±0.103	0.60±0.174	0.97
IgG, mg/ml	2.15±0.230	2.28±0.356	2.40±0.222	2.77±0.343	2.4*
Immune potency, %	100	100	100	100	

Note: * – level of reliability (* – P>0.01).

Based on Table 8, in 20–21-day-old embryos, the immunobiological parameters of blood serum remained at a high level, indicating that the immune system had reached its final functional maturation stage.

Total protein ranged from 4.62 to 4.85 g%, with an average of 4.76 g%. Values remained stable throughout the day, reflecting a balanced protein metabolism.

Albumin ranged from 1.59 to 1.95 g%, averaging 1.79 g%. The highest value was recorded at 18:00 (1.95±0.192 g%), indicating adequate liver synthetic function.

α-globulins varied between 0.40–0.82 g%, averaging 0.632 g%, with the minimum at 18:00 (0.40±0.022 g%).

β-globulins showed an increasing trend from 0.62 to 0.88 g%, averaging 0.77 g%, peaking at 24:00.

γ-globulins ranged from 1.32–1.59 g%, averaging 1.452 g%, reflecting active immunoglobulin synthesis.

IgM ranged from 0.60–1.28 mg/ml, averaging 0.97 mg/ml. The peak at 18:00 (1.28±0.103 mg/ml) demonstrates a highly active primary immune response.

IgG ranged from 2.15–2.77 mg/ml, averaging 2.4 mg/ml, with the highest value at 24:00 (2.77±0.343 mg/ml, *P<0.05), indicating a stable and robust humoral immunity.

Immune capacity remained at 100% across all time points, confirming maximal immune reactivity and full functional development of the defense system.

During the 20–21-day embryonic period, the high levels of γ-globulins, IgM, and especially IgG indicate the full formation of humoral immunity. The immune capacity reaching 100% confirms that the organism is highly resistant to external factors. This stage marks the functional stabilization and maturation of the immune system.

Notably, albumin levels showed a statistically significant decrease at the final stage of embryogenesis (*P<0.05). According to literature, if maternal IgM concentrations are high, IgM passes into the egg white during ovogenesis. At the final stage of embryonic development, these immunoglobulins are absorbed into the embryo via pinocytosis in the

intestines, explaining the detectable IgM levels in the blood serum, as illustrated in Tables 5–9.

As seen from Table 9, in 1-day-old chicks, the immunobiological indicators in the blood serum were recorded at a high level, which is primarily associated with passive immunity acquired from the mother.

The total protein content ranged from 4.61 to 4.94 g%, with an average of 4.787 g%. A slight decrease was observed during the day, which can be explained by postnatal adaptation processes.

Table 9. Dynamics of immunobiological indicators in the blood serum of 1-day-old chicks, M±m (n=10).

Parameters	Chronology of Blood Sampling During the Day (hours)				
	6	12	18	24	Avarage
Total protein, g%	4.94±0.430	4.86±0.540	4.74±0.338	4.61±0.684	4.787
Albumins, g%	1.80±0.168	1.70±0.166	1.63±0.200	1.61±0.241	1.685
α-globulins, g%	0.35±0.029	0.38±0.032	0.41±0.036	0.38±0.054	0.38
β-globulins, g%	0.54±0.044	0.47±0.038	0.45±0.042	0.37±0.065	0.457
γ-globulins, g%	2.29±0.115	2.39±0.180	2.29±0.166	2.28±0.33	2.312
IgM, mg/ml	1.18±0.168	1.35±0.105	1.35±0.100	1.52±0.193	1.35
IgG, mg/ml	6.29±0.888	6.34±0.646	7.29±0.996	6.65±0.949	6.642*
Immune potency, %	100	100	100	100	

Note: - *level of reliability (*- P>0.01).

Albumin levels ranged from 1.61 to 1.80 g%, averaging 1.685 g%. The gradual decline reflects metabolic readjustment processes.

α-globulins ranged from 0.35 to 0.41 g% (average 0.38 g%) and remained relatively stable.

β-globulins ranged from 0.37 to 0.54 g%, with an average of 0.457 g%. A slight decrease was observed throughout the day.

γ-globulins were very high, ranging from 2.28 to 2.39 g%, with an average of 2.312 g%. This indicates the presence of maternal immunoglobulins in the newly hatched chicks.

IgM levels ranged from 1.18 to 1.52 mg/ml, with an average of 1.35 mg/ml, showing a tendency to increase during the day.

IgG levels ranged from 6.29 to 7.29 mg/ml, with an average of 6.642 mg/ml, and were statistically significant (*P<0.05). This demonstrates a high level of passive humoral immunity.

Immune competence was 100% at all time points, indicating that the protective mechanisms in newly hatched chicks were at their maximum.

In the 1-day postnatal period, the high levels of γ-globulins and especially IgG in chicks are associated with maternal passive immunity. In subsequent age periods, this passive immunity gradually decreases, being replaced by active (self-generated) immunity. This stage represents the period of adaptation to the external environment and the transition of the immune system to independent function (Table 9).

3.1 Dynamics of Immunobiological Indicators in Blood Serum and Passive Immunity Against Newcastle Disease Pathogen in Chicks During the First Week of Postnatal Development

As noted in the scientific literature, the formation of passive immunity continues during postnatal development. This is because the absorption of immunoglobulins present in the

yolk sac, which are swallowed into the gastrointestinal tract, persists even after the chicks hatch.

To clarify this process, the immunobiological indicators of blood serum were studied in chicks from hatching until they reached one week of age. The experiment involved 70 chicks hatched within one hour of each other. Every day, blood was collected from 10 chicks. For one-day-old chicks, blood samples were taken during the first hour of life, while for the remaining chicks, blood was collected at 23:00, prior to turning off the lights for a short rest period. The results are presented in Table 10.

As shown in Table 10, the total protein content in the blood serum of hatched chicks increased over time. However, this increase reached a statistically significant level only in three-day-old chicks ($P < 0.05$).

The elevated total protein observed in three-day-old chicks began to decrease from day six and corresponded to the levels recorded in seven-day-old chicks ($P > 0.05$). Albumin levels also started to rise from day three, though they did not reach statistical significance ($P > 0.05$).

The α-globulin fraction remained stable during the first six days but showed a slight decrease on the final day ($P < 0.05$). A transient decrease in β-globulin levels was observed in three- to four-day-old chicks ($P < 0.05$).

In contrast, γ-globulin levels were considerably higher during this period. Throughout the remainder of the first postnatal week, γ-globulin concentrations remained relatively elevated ($P < 0.05$).

Thus, during the first week of postnatal development, the dynamics of immunoglobulin levels in chick blood serum exhibited a distinct pattern. Specifically, IgM concentrations sharply increased from day three and remained elevated on day five ($P < 0.05$). However, by day six, IgM levels began to decline and reached a statistically significant decrease by day seven.

IgG concentrations remained relatively stable without significant differences during the first four days. From day five onwards, IgG levels gradually increased, reaching statistically significant values by days six and seven ($P < 0.05$).

Table 10. Relationship between immunobiological indicators in blood serum and passive immunity parameters during the first week of postnatal development in chicks, M±m (n=10).

Parameters	Postnatal development days							
	1	2	3	4	5	6	7	Avareg e
Total protein, g%	4.76±0.56	4.91±0.645	5.28±0.388*	5.49±0.546	5.23±0.648	4.88±0.764	4.79±0.72**	5.048
Albumins, g%	1.48±0.186	1.45±0.224	1.53±0.136**	1.67±0.203	1.74±0.176	1.61±0.189	1.71±0.228	1.598
α-globulins, g%	0.62±0.054	0.74±0.038	0.67±0.067	0.73±0.092	0.81±0.090	0.75±0.087	0.49±0.098*	0.681
β-globulins, g%	0.53±0.066	0.65±0.042	0.38±0.63*	0.41±0.064*	0.55±0.086	0.44±0.046	0.52±0.071	0.497
γ-globulins, g%	2.13±0.24*	2.07±0.28*	2.70±0.18*	2.58±0.16*	2.13±0.22*	2.08±0.12*	2.07±0.32*	2.251*
IgM, mg/ml	1.47±0.132	1.66±0.136	2.14±0.280	2.26±0.196	1.97±0.178*	1.83±0.180	1.46±0.261*	1.827
IgG, mg/ml	7.10±1.09	7.28±1.14	7.10±1.10	7.29±1.76	7.48±0.97	7.78±0.98*	7.90±1.06*	7.418
Immune potency, %	100	100	100	100	100	100	100	

Note: - *significance level (*- P<0.05, **- P>0.05).

It was observed that during the early postnatal life of chicks, the level of immunity in the blood serum against the Newcastle disease virus remained high and stable.

A key theoretical consideration is that antigens, including anti-immunoglobulins, when introduced into the organism solely for their immunogenic effect, may also induce immunological tolerance, a factor often overlooked. According to literature, ontogenetic data on autoantigens' ability to induce immunological tolerance have not yet been fully

applied in immunoprophylaxis practice. However, experiments conducted by the authors on chicken embryos demonstrated the ontogenetic timing at which immunological tolerance is established, though the role of this process in passive and active immunity formation has often been neglected. Without accounting for this, the role of passive and active immunity remains incomplete.

Consequently, conflicting results regarding the influence of passive immunity on the development of active immunity have been difficult to resolve. The conducted studies clarified these contradictions: if chicks with a high level of passive immunity are vaccinated, the formation of active immunity post-vaccination is hindered, whereas vaccinating chicks when passive immunity is declining facilitates active immunity development.

However, since many scientific sources report only one side of this contradiction, traditional interpretations have limited the negative impact of high-titer passive immunity on post-vaccination immune responses solely to the neutralization of vaccine antigens by maternal antibodies (interference). In our view, this explanation is incomplete because anti-idiotypic antibodies synthesized in response to antigenic stimulation simultaneously exert immunogenic effects alongside vaccine antigens, generating an immunological resonance phenomenon that enhances post-vaccination immune responses. Conversely, when passive antibody titers are high, anti-idiotypic antibodies do not accumulate, and part of the vaccine antigen is neutralized, which also influences the observed immune response.

Evidence supporting this conclusion comes from comparing vaccination outcomes in chicks with passive immunity below 80% to those in control chicks. Indeed, according to current recommendations, chicks are usually vaccinated against Newcastle disease at 14–15 days of age, when passive immunity has sufficiently declined. However, this does not clarify whether the diminished passive immunity directly facilitates active immunity or whether other factors are involved.

Our research demonstrated that the phenomenon is not solely due to antibody interference: monitoring post-vaccination immunity in chicks with passive immunity above 80% cannot be explained solely by neutralization effects. Therefore, maternal antibodies themselves may exert an independent positive effect, acting not only as antibodies but also as antigens.

Utilizing the antigenic properties of immunoglobulins and the anti-immunoglobulins they elicit (anti-idiotypic antibodies) allows harnessing the immunological resonance phenomenon, significantly enhancing the effectiveness of immunoprophylaxis. Furthermore, immunoglobulins constituting passive immunity contribute not only to humoral defense but also facilitate active immunity via anti-idiotypic antibodies, engaging the cellular immune system and thereby helping prevent epizootics and panzootics in wild animals.

Indeed, although avian influenza spreads via wild birds, the process rarely escalates to epizootic or panzootic levels because immune protection, selected via immunological receptors (including anti-idiotypes) and maintained by macrophage and lymphocyte populations, serves as a preventive factor.

Thus, the formation of passive immunity during natal and early postnatal development is not merely a transient event. Immunoglobulins in passive immunity act not only as antibodies but also as antigens, participating in the induction of active immunity.

The delayed absorption of gamma-globulins, especially IgM, during postnatal development, when the organism's tolerance to foreign antigens has declined, allows native immunoglobulins to function both as antibodies and antigens. The increase in IgM absorption from the final days of embryogenesis, despite not reflecting in GATR titers, and the concurrent decrease in total serum protein, indicates that a significant portion of immunoglobulins participates in receptor formation on macrophage surfaces.

Although egg-derived proteins initially enter the chick's blood under tolerant conditions (without eliciting an immunogenic effect), the observed trends in the first postnatal week, particularly the statistically significant increase in IgM in 3–7-day-old chicks, indicate that IgM absorbed via pinocytosis in the intestine acts as an antigen, inducing an immune response.

Additionally, fluctuations in total serum protein during this period, without corresponding changes in GATR titers, further support this conclusion.

The dynamics of serum protein fractions and immunoglobulin types during the first week postnatally also reveal the role of passive immunity: while total protein, gamma-globulin, and IgG levels remained stable and high, IgM levels increased significantly from days 3–6 ($P<0.01$), without corresponding fluctuations in α- and β-globulins ($P>0.05$). This underscores IgM as a key factor in transforming passive immunity into active immunity.

IgM immunoglobulins are pentameric molecules, each subunit containing two antigen-binding sites (molecular mass ~990 kDa, half-life ~5 days). IgM binds complement more effectively than IgG, agglutinates bacteria, neutralizes viruses, and activates phagocytosis, playing a critical role in early infection control.

Following antigen exposure, immunoglobulins are produced sequentially: IgM first, followed by IgG, then IgA. In serum, 70–90% of immunoglobulins are IgG, 10–15% IgA, 5–10% IgM, and only ~0.2% are IgD or IgE. IgM's early production and large molecular structure are essential for controlling widespread (pantropic) infections. High antigen loads result in abundant antibody production, sustaining immunity.

Therefore, in controlling pantropic infections, increasing IgM concentration is crucial.

Finally, data from the "Ohalik-Lohmann" poultry farm vaccination monitoring, combined with embryonic and chick experiments and trials testing the immunogenicity of anti-idiotypic antibodies, confirm that passive immunity is not limited to the first postnatal week but continues to influence active immunity development.

4 Conclusion

Based on the results of the study, we obtained the following conclusions:

During the last three days of postnatal development, IgM immunoglobulins are absorbed by the chick embryo through the intestines via pinocytosis, and maintaining these immunoglobulins at high concentrations is of significant immunological importance.

For effective immunoprophylaxis against pantropic viral infections (Newcastle disease, avian influenza, infectious bronchitis), ensuring the synthesis of high levels of IgM antibodies is a key immunological measure.

At the onset of postnatal development, achieving high-level synthesis of IgM antibodies requires a substantial presence of passive IgM immunity.

To ensure a high level of passive immunity in chicks, it is necessary to establish a high level of active immunity in the mother hens.

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