DOI: https://doi.org/10.21323/2414-438X-2022-7-3-193-199

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

Received 19.08.2022
Accepted in revised 05.09.2022
Accepted for publication 21.09.2022

CONTRIBUTION OF SOME IMMUNOLOGICAL AND METABOLIC FACTORS TO FORMATION OF PIGLETS' POST-VACCINATION IMMUNITY

Marina A. Derkho [1], Pavel V. Burkov [1], Pavel N. Shcherbakov [1], Maksim B. Rebezov [2,*], Ksenia V. Stepanova [1], Arif N. M. Ansori [3]
[1] South Ural State Agrarian University, Troitsk, Russia
[2] V.M. Gorbatov Federal Scientific Center of Food Systems, Moscow, Russia
[3] Universitas Airlangga, Surabaya, Indonesia

Keywords: *piglets, post-vaccination immunity, natural resistance factors, biochemical parameters*

Abstract
The role and responsibility of natural resistance factors, protein and lipid metabolism in the formation of piglets post-vaccination immunity against circovirus is researched. Blood was taken for tests before and on the 15th, 40th and 70th day after the vaccination. The sampled blood was analyzed to determine immunological and biochemical parameters. It was revealed that before vaccination, 31.46% of the studied samples have a positive reaction in ELISA; their number increases to 67.80–71.16% on the 40th and 70th days after vaccination.In the blood of piglets, especially on the 40th and 70th day after the vaccination, the total count of leukocytes, monocytes and lymphocytes increases by 1.21; 2.28 times and 1.48 times, but neutrophils reduced by 1.74 times along with the phagocytic properties activation. The anabolic directivity of protein metabolism is defined by the synthesis of globulin proteins. At the same time albumin-synthesizing activity in a liver decreased and "cytolysis reaction" of hepatocytes was detected. In the lipid profile of piglets' blood, the content of LDL-cholesterol increased by 1.44 times, while that of triglycerides decreased by 2.64 times. X-ray spectral analysis revealed the correlation between the formation of post-vaccination immunity and two factors: the factor of the principal component (PC) 1, which is predominantly associated with indicators of natural resistance, and PC2, which is associated with metabolism indicators. The research results show that in order to increase the efficiency of formation of post-vaccination immunity in piglets, it is necessary to combine vaccination with hepatoprotective drugs.

For citation: Derkho, M.A., Burkov, P.V., Scherbakov, P.N., Rebezov, M.B., Stepanova, K.V., Ansori, A.N.M. (2022). Contribution of some immunological and metabolic factors to formation of piglets' post-vaccination immunity. *Theory and Practice of Meat Processing*, 7(3), 193-199. https://doi.org/10.21323/2414-438X-2022-7-3-193-199

***Funding*:**
The work was supported by the grant No. 19–16–00068-P 22–16–20007 of the Russian Science Foundation.

Introduction

Vaccination is the main way of struggle against the most infectious diseases, including porcine circovirus of type 2 (PCV2), which is possible due to the availability of specific vaccines [1]. The vaccine, after being introduced into the body of animals, stimulates the immune system due to the "launch" of the humoral and cell-mediated immune response. In this case the humoral immune response is provided not only by the synthesis of antibodies, which neutralize the virus, but also is stipulated by antibodies that stimulate the complement system and antibody-dependent cellular cytotoxicity. Cellular immunity factors activate cytotoxic T-cells and macrophages [2].

However, vaccination does not always provide a sufficient level of "herd immunity" necessary to protect animals in large industrial farms due to the quick "moving" of the animals during the technological cycle, as well as due to the low immunological response of pigs, caused by flaws and drawbacks in technology of their managing and feeding, but most importantly — by the circulation of viruses in industrial premises as the viruses feature high resistance in the environment.

Therefore, despite regular vaccination, the infectious agent still circulates in the farm facilities, thus reducing the immune response of animals. At the same time, various respiratory co-infection very often joins the virus and initiates the symptoms complex, known as "the disease associated with porcine circovirus" [3,4]. Piglets are most sensitive group of animals to this complex of porcine respiratory diseases during the period of growing up till fattening period [4]. The joint disease provides a negative impact on the rate of growth and development of the animals, rate of feed digestion; it increases the rejection rate of animals, leading to economic losses of the farms [5,6,7,8]. So, the research of the patterns of formation of post-vaccination immunity in piglets is one of the urgent and relevant issues in pig breeding.

Most researches on this issue represent the results of testing the efficiency of vaccines against the virus [4,9,10]. Meanwhile, the number of researches that reveal the mechanisms of formation of post-vaccination immunity against

PCV2 is quite insignificant. In particular, it was found that in the body of vaccinated pigs, the humoral and cellular immune response factors feature the greatest variability. At the same time, cytotoxic T-cells, T-helper cells of the 1st (Th1) and 17th type (Th17) are most strongly activated, reducing the viral load on the body of vaccinated pigs [11]. It was noted in studies [11,12] that vaccination is a stress factor for the animal's body. Therefore, during the post-vaccination period the changes, typical for alert stage, develop in the body of animals. These changes are accompanied by fluctuations in proteins levels during the acute phase of inflammation, variations of glucocorticoids content in the blood [12,13], immunosuppression [14], changes in antioxidant activity and metabolism of lipids and proteins [15], thus affecting the "quality" of the forming immunity. At the same time, the immune memory is modulated by the influence of a large number of factors, which diversely contribute to maintaining the titer of specific antibodies [16,17]. Thus, according to the research [18], the efficiency of formation of post-vaccination immunity is associated with such a parameter as the growth rate of piglets, which correlates with the degree of lung damage. As is known, the intensity of growth processes is interrelated with the metabolic status of the body, which provides the cells of the body with macronutrients and energy [19,20]. Therefore it is possible to form an "adequate" immune response after vaccination in conditions of maintaining the activity and usual metabolic processes in the growing body of piglets.

In addition, it has been established that due to the "antigenic drifting of circulating viruses", most vaccines need constant renewal. Only in this case they provide sufficient protection of the animal organism after its vaccination [2]. However, renovating a vaccine is quite a labor-consuming process; it cannot keep up with the rate of emergence of circulating strains of viruses, especially in the specific conditions of a region and the certain enterprise. Therefore, vaccines against most animal respiratory diseases are remade very rarely, which does not provide complete protection against the current type of infection [21]. For these reasons, it is important to expand the body's immune response to vaccination, and for this it is necessary to study the patterns of formation of the post-vaccination immunity, including not only immunological, but also metabolic parameters. This will enable us to form a "vaccination strategy" that maximizes the efficiency of post-vaccination immunity.

The purpose of this research was to assess the contribution of natural resistance factors, parameters of protein and lipid metabolism in the body of piglets in the formation of post-vaccination immunity against circovirus.

Materials and Methods

Ethical statement

This study was planned and run in accordance with the recommendations of the bioethics committee of the South Ural State Agrarian University (Chelyabinsk region, Russia), and was also agreed upon with the veterinary service of the agricultural company.

Animals, Study Design

The on-site part of the research was run in 2022 at the agricultural company "Ariant" LLC (Chelyabinsk region). An experimental group (n = 3618) of suckling piglets was taken at the one of the pig farms of the agricultural company. This pig farm specializes in growing of commercial young pigs, which were vaccinated with the Ingelvak CircoFLEX vaccine (Germany) against circovirus on the 21st day of life in accordance with the vaccine manufacturer's recommendations. At the age of 23–24 days, the piglets were taken away from their breeding sow and transferred to the nursery. There they were kept in group cages of 20–25 heads each. The cages were equipped with freely accessible automatic feeders and waterthroughs. The technology of feeding and managing the animals complied with recommendations of *Genesis*. The nutrients of regional origin were used for the compound feed production.

Data collection

To conduct immunological and biochemical studies in 5% of the animals of the experimental group, blood samples were randomly taken from the *vena cava cranialis* with the help of VACUETTE vacuum-smart tubes (9ml, 16x100mm), Zhejiang Gongdong Medical Technology Co. Ltd. (9 ml,16x100mm), with the double ended blood sampling needles 14Gx3–1/4 (2.1x80mm).

Whole blood was used to determine: 1) the number of leukocytes, lymphocytes and monocytes on a Mindray BC2800 Vet hematological analyzer (China) with species-specific settings for pigs; 2) phagocytic activity of neutrophilic granulocytes. To obtain leukocyte suspension, blood samples were incubated at 37 °C at an angle of 45° for 40 min, this way initiating spontaneous sedimentation of erythrocytes. As a microbial test object, a suspension of a day-aged culture of *Escherichia coli* was used (1 billion cells in 1 ml of the suspension). Microbial suspension and leukocyte suspensions were mixed in strips at a ratio of 1:1, and incubated in a thermoshaker (Elmy, Latvia) at 37 °C for 40 minutes and centrifuged. The smears were prepared from the precipitate on defatted glass slides and stained according to the method of Romanovsky-Giemsa. The result of phagocytosis was assessed on 100 cells with an immersion system of a light microscope and a magnification of 10x90. Phagocytic activity of neutrophils (PAN,%) was calculated as a percentage of phagocytic cells in reference to their total number (100 cells); phagocytic number (PN, c. u.) was calculated as an average number of phagocytosing microbes captured by one active neutrophilic granulocyte; phagocytic index (PI,%), as a percentage of the average number of phagocytosed microbes in reference to the total number of neutrophilic granulocytes (100 cells).

Blood serum obtained after blood clot sedimentation was used: 1) to determine specific antibodies to circovirus

using CIRCOSerotest kits (LLC Vetbiokhim, Russia) by immunoenzyme method. The analysis was run in accordance with the instructions for the test kit provided by the manufacturer. The result was expressed as a percentage. If the ratio of conjugate binding (Rcb,%) with blood serum antibodies exceeded 20%, then it was considered positive; 2) to determine biochemical parameters (total protein, albumin (A), urea, ALT and AST activity) using a Super Z biochemical analyzer (China). Additionally, the calculation method was used to determine the concentration of globulins (G = Total protein (g/l) — albumins (g/l)), ration of proteins (albumins (A, g/l) / globulins (G, g/l)), ratio between total protein (g/l) and urea (mmol/l).

Statistical analysis

Statistical analysis was run by the software Statistica 6.0. The significance of the differences was set at $p < 0.05$. All laboratory data were expressed as mean (X) and standard error of the mean (Sx). The principal component analysis (PCA) was used to determine the blood parameters, most significantly associated with the titer of antibodies to virus antigens [22]. The correlation of those parameters was judged by the values of the correlation indices determined by Spearman method between the indicator and its load on the principal component (PC). The number of principal components was determined by the graphical method of Cattell's scree [23].

Results and discussion

Vaccination against PCV-2 reduces the viral load on the body of piglets and prevents specific damage to lymphoid tissue due to production of specific antibodies [24]. The formation of an immune response in the organism of animals was tested by the seropositivity of the piglets, determined by ELISA kit (Table 1).

Table 1. Results of detection of antibodies (IgG) to PCV-2 by enzyme-linked immunosorbent assay

Age of the piglets	After vaccination, days	Number of piglets with a positive ELISA test, %	Rcb, %
19 days	Before vaccination (background)	31.46	39.34 ± 0.64
35 days	15 days	33.63	41.56 ± 1.29
60 days	40 days	67.80	78.54 ± 1.85
90 days	70 days	71.16	24.35 ± 0.68

The results of our research showed that 31.46% of suckling piglets had a positive ELISA test before vaccination. The count of IgG antibodies, estimated by Rcb, was equal to 39.34 ± 0.64% (Table 1).

In the post-vaccination period, the number of animals in the experimental group with a positive ELISA test increased, and in the period 40–70 days after vaccination, it fluctuated in the range of 67.80–71.16% of their total number of studies performed.

However, the dynamics of detection of positive ELISA samples did not correspond to the results of quantitative analysis.

So, on the 15th day after vaccination the Rcb practically did not differ from the background values; its peak was observed on the 40th day, exceeding the background value by 2.00 times (78.54 ± 1.85%). By the end of the rearing period, the Rcb decreased to a borderline value, although the number of piglets with a positive ELISA test remained practically unchanged.

To define the responsibility of some blood parameters in the formation of post-vaccination immunity, we researched their dynamics during the growing period, as this period is the most "critical" in terms of animals' rejection in the pig-breeding complex environment.

It is possible to judge indirectly on the status of cell-mediated immune responses in the body of piglets in the post-vaccination period by the variability of indicators of the natural resistance [25]. Thus, the total number of leukocytes in the blood of animals, which characterizes the immune potential of the body, increased systematically in the post-vaccination period, reaching its maximum in piglets of 60- and 90-days old (Table 2). At the same time, the main changes in their group were observed on the 40th day and 70th day after vaccination. At the control point — "the 40th day after vaccination" — the changes were most profound in comparison with the background; they manifested themselves in the form of a sharp increase of lymphocytes number (by 1.48 times, $p < 0.05$) and neutrophils decrease (by 1.74 times, $p < 0.05$), and on the 70th day we observed an increase in the number of lymphocytes (by 1.17 times, $p < 0.05$), monocytes (by 2.28 times, $p < 0.05$) and neutrophils (by 1.25 times $p < 0.05$). < 0.05).

The phagocytic properties of neutrophils weren't correlated with changes in their number in the blood of piglets. The absorption function of cells, assessed by the phagocytic activity of neutrophils, phagocytic number and phagocytic index, was profound on the 40th and 70th days after vaccination, exceeding the values "before vaccination" by 13.11–20.12; 67.51–83.24 and 31.22–39.29%, respectively (Table 2).

The performance of the vaccine immunogenic properties was also represented in the metabolic status of the growing piglets. In our research we assessed the protein and lipid metabolism, most of the indicators of which in the post-vaccination period were varying within the normal range. Thus there was a trend towards building-up the total protein concentration in the piglets' blood by 17.52% (Table 2) due to an increase of globulins share (by 33.99%) and a decrease of albumins share (by 9.92%). This way the ration of proteins (A/G) decreased by 33.33% ($p < 0.05$). The positive age-related dynamics of total protein value was caused by systematic decrease of urea level in the blood by 36.39%. This fact proves retention of protein nitrogen in the body of piglets and shows an increase in ratio of total protein/urea by 1.84 times ($p < 0.05$). However, AlAT

Table 2. Immuno-metabolic parameters of piglets' blood (X ± Sx)

Parameter	Age of the piglets / time period after vaccination, days			
	19 / before vaccination	35/15	60 / 40	90 / 70
Indicators of the natural resistance				
Leukocytes, 10^9/l	19.31 ± 0.87	21.31 ± 0.98	23.19 ± 0.77*	23.38 ± 0.46*
Lymphocytes, 10^9/l	12.22 ± 0.61	13.81 ± 0.94	18.15 ± 0.37*	14.34 ± 0.60*
Monocytes, 10^9/l	0.66 ± 0.02	0.45 ± 0.11*	0.69 ± 0.07	1.51 ± 0.11*
Neutrophils, 10^9/l	6.01 ± 0.37	6.44 ± 0.56	3.75 ± 0.55*	7.53 ± 0.24*
PAN (phagocytic activity of neutrophils), %	40.04 ± 0.90	42.81 ± 0.79	48.10 ± 0.42*	45.29 ± 0.31*
PN (phagocytic number), c. u.	1.97 ± 0.12	2.21 ± 0.25	3.61 ± 0.12*	3.30 ± 0.22*
PI (phagocytic index), %	2.85 ± 0.17	3.01 ± 0.25	3.97 ± 0.32*	3.74 ± 0.26*
Metabolic indicators				
Total protein, g/l	64.50 ± 1.50	65.30 ± 1.99	69.40 ± 0.70	75.80 ± 0.49*
Albumins (A), g/l	24.20 ± 1.30	22.70 ± 1.01	22.40 ± 1.10	21.80 ± 1.80
Globulins (G), g/l	40.30 ± 0.54	42.60 ± 0.49	47.00 ± 0.32*	54.00 ± 0.61*
A/G, c. u.	0.60 ± 0.03	0.53 ± 0.06	0.48 ± 0.05*	0.40 ± 0.03*
Urea, mmol/l	5.66 ± 0.78	4.67 ± 0.34	3.96 ± 0.23*	3.60 ± 0.21*
Total protein/urea, c. u.	11.39 ± 1.20	13.98 ± 0.36*	17.52 ± 0.56*	21.06 ± 1.10*
AsAT, mmol/l · h	1.57 ± 0.08	1.44 ± 0.10	1.29 ± 0.11	0.66 ± 0.13
AlAT, mmol/l · h	1.53 ± 0.12	2.19 ± 0.15*	2.11 ± 0.11*	1.63 ± 0.15
Total lipids, g/l	2.93 ± 0.15	2.67 ± 0.12	2.27 ± 0.07*	2.42 ± 0.16*
Cholesterol, mmol/l	2.61 ± 0.14	2.37 ± 0.13	2.41 ± 0.11	2.87 ± 0.17
LDL-cholesterol, mmol/l	1.10 ± 0.10	1.26 ± 0.08	1.49 ± 0.07*	1.58 ± 0.14*
Triglycerides, mmol/l	0.37 ± 0.04	0.31 ± 0.03	0.34 ± 0.03	0.14 ± 0.01**

Note: * — $p < 0.05$ to the "before vaccination (background)" value.

activity increased by 1.36 times ($p < 0.05$), exceeding the limits of the norm, and AsAT, on the contrary, decreased by 2.37 times ($p < 0.05$), but corresponded to its limits. This means that the body of piglets in the post-vaccination period demonstrated an imbalance in the synthesis of proteins and the directivity of amino acids participation in metabolic processes.

More significant changes were noted in lipid metabolism (Table 2): the concentration of total lipids and triglycerides in the piglets' blood decreased by 1.29 and 2.64 times ($p < 0.05$), but the level of total cholesterol and LDL cholesterol increased by 1.21 and 1.43 times ($p < 0.05$).

To determine the parameters of the sampled blood which are most significantly associated with the process of immunity formation in the post-vaccination period, we used the principal component analysis (PCA) [26]. While "compressing" the identified multidimensional correlations using the method of Cattell [23], two most significant factors were determined: the main component 1 (PC-1) and the main component 2 (PC-2), which components determine more than 70% of variance of features in the statistical matrix. Further, the identified correlations were ranked in the context of the main components according to the strength of the relationship during the post-vaccination period (Table 3).

At the same time, GC-1 had a predominant associativity with indicators of natural resistance, and GC-2 — is associated with metabolic indicators, which content in the blood is directly or indirectly related to liver functions [19]. Consequently, the process of formation of post-vaccination immunity in the body of piglets is determined not only by changes in its immunological status, but by their metabolic status too.

Table 3. Associativity of the main components with blood parameters

Parameters	Age of the piglets / time period after vaccination, days					
	35/15		60 / 40		90 / 70	
	PC1	PC2	PC1	PC2	PC1	PC2
Indicators of natural resistance						
Leukocytes, 10^9/l	++	+	++	+	+++	+
Lymphocytes, 10^9/l	+++	+	+++	++	+++	++
Monocytes, 10^9/l	+++	+	++	+	+++	+
Neutrophils, 10^9/l	+++	+	+++	+	+++	++
PAN (phagocytic activity of neutrophils), %	++	+	++	+	++	+
PN (phagocytic number), c. u.	++	+	++	+	++	+
PI (phagocytic index), %	++	+	++	+	++	+
Metabolic indicators						
Total protein, g/l	+	++	+	++	+	
Albumins (A), g/l	+	+++	+	+++	+	+++
Globulins (G), g/l	++	++	++	+++	+++	+++
A/G, c. u.	+	++	++	+++	+	+++
Urea, mmol/l	+	+++	+	+++	+	+++
Total protein/urea, c. u.	+	++	+	+++	+	++
AsAT, mmol/l · h	++	+++	+	+++	++	+++
AlAT, mmol/l · h	+	+++	+	+++	++	+++
Total lipids, g/l	+	++	+	++	+	++
Cholesterol, mmol/l	+	++	++	+++	+	+++
LDL-cholesterol, mmol/l	+	+++	+	+++	+	+++
Triglycerides, mmol/l	+	+++	+	++	+	++

Note: the significance of correlations intensity of between the parameters: "+" — r=0.3–0.5; "++" — r=0.5–0.7; "+++" — r=0.7 and more

The global trade of breeding pigs, semen, and pork products has contributed to the worldwide extension of PCV-2 [5]. In order to prevent the disease the animals undergo the vaccination. For vaccination various commercial vaccines are used. In our research we used the vaccine Ingelvak CircoFLEX (Germany), which contributed to the emergence of antibodies in the body of animals. These antibodies bind and neutralize the virus and prevent its further spread [24]. We found that 31.46% of suckling piglets had specific antibodies to PCV-2 before their vaccination as a result of their passive immunization under conditions of a controlled subclinical course of infection [27]. The presence of the antibodies in the first 15 days after vaccination inhibited the formation of a humoral immune response. This inhibition was caused by their influence on the cell-mediated and antibody-mediated mechanisms of immunity in the body of piglets [28]. Studies [29] also confirmed the low PCR-positivity of piglets in the early stages of the post-vaccination period. However, on the 40th day after vaccination, the ration of conjugate binding by antibodies increased dramatically from 39.34 ± 0.64 up to 78.54 ± 1.85%, although the number of samples with a positive ELISA sample was 67.80% of the total number of studies performed. Consequently, passively acquired antibodies influenced the formation of a post-vaccination immune response in piglets [27]. Therefore, the number of positive samples on the 70th day after vaccination practically did not change (71.16%), and the value of Rcb decreased to 24.35 ± 0.68%, that is, almost to the threshold value of a positive test in the ELISA method used.

In general, it can be stated that the number of positive samples in the post-vaccination period systematically increased as a result of the development of specific antibodies to PCV2, but the intensity of post-vaccination immunity formation was related to presence of antibodies against the virus in the body of animals before immunization.

Post-vaccination variability of leukocyte cells in the body of piglets was determined by their participation in the processes of opsonization, transfer and phagocytosis of antigenic particles [30]. Thus, the increase in the total count of leukocytes proved the increase of the body reactivity. The redistribution of cells in the leukocyte pool, especially in 40 days after vaccination, indicated the activation of leukocytes proliferation processes and their release from the organs of leukopoiesis into the bloodstream [31]. At the same time, the variability of lymphocytes corresponded to fluctuations of monocytes, which was consistent with coordination of the mechanisms for formation of their pool in the piglets' blood after vaccination [32]. Meanwhile the functions of lymphocytes and monocytes in production of neutralizing antibodies were reflected both in the count of circulating neutrophilic granulocytes and their phagocytability.

Currently there are a row of researches that have revealed the effect of vaccination on the metabolic status of the liver [33], which is related to the hepatodepressive effect of the vaccine. Meanwhile, the antigenic components of the vaccine and the products of their seroconversion are found not only in the organs of immunogenesis, but are also found in the liver [34] as the central metabolic organ [19,20].

Blood proteins play an important role in the homeostatic balance of the body. In the post-vaccination period the count of total protein in the piglets' blood increases due to globulin fractions, which contain significant share of gamma globulins, involved in the formation of the body's immune response [19]. However, under the conditions of the anabolic directivity of protein metabolism in the blood, the count of albumins, synthesized only in liver cells and being a marker of its protein-synthesizing function, decreased [35]. At the same time, AlAT activity increased as a result of intensification of hepatocytes cytolysis reaction [20]. Consequently, in the post-vaccination period metabolic changes were observed in the liver. Those changes are probably associated with lymphocyte-mediated immune responses of cytolysis [36], or vaccine-induced or mediated immune response of hepatocytes to administration of antigen [37].

During the post-vaccination period the blood lipid profile of piglets also changed. The peak of changes was found in level of LDL-cholesterol and triglycerides. It determined the risk of cardiovascular changes in the body of an animal [38], as well as it testifies on mutation in directivity of lipid metabolism in liver cells.

Based on the fact that the body of a piglet is a multi-parameter system [39], we tried to minimize the amount of initial laboratory information by excluding data that are not directly related to the formation of immunity after vaccination. Using the PCA method, we determined two priority factors: the principal component 1 (PC1) and the principal component 2 (PC2), in the context of which the associativity of blood parameters was determined. Thus, in the post-vaccination period in the context of PC1, the indicators of natural resistance turned out to be interrelated and mutually correlated variables. Their variability was directly associated with the formation of the animals' seropositivity [28]. As far as PC2 is concerned, here, on the contrary, associativity with metabolic parameters was revealed, which parameters should be attributed to "indirect" blood parameters, reflecting both the metabolic functions of the liver and the adaptive potential of the animal's organism [19]. Consequently, the efficiency of immunological reactions is not only associated with factors of cellular and humoral immunity, but also with factors that determine the provision of immune processes with macronutrients.

Conclusion

The results of the work showed that before vaccination, 31.46% of blood samples of piglets have a positive reaction in ELISA. After vaccination, their number increases to 67.80–71.16%, although the quantitative expression of the conjugate binding coefficient is 78.54 and 24.35%, respectively. In the post-vaccination period, the indicators of the body's natural resistance change in accordance with its role in immunological reactions and

manifest themselves most intensively on the 40th and 70th days after the vaccination. Meanwhile, the total count of leukocytes in the animals' blood increases by 1.21 times ($p < 0.05$), of monocytes by 2.28 times ($p < 0.05$), lymphocytes by 1.48 times ($p < 0.05$), especially on the 40th day after the vaccination, but the count of neutrophils decreases by 1.74 times ($p < 0.05$), although phagocytic properties increase (phagocytic activity of neutrophils, phagocytic count and phagocytic index by 13.11–20.12; 67.51–83.24 and 31.22–39.29%, respectively). In the post-vaccination period the protein metabolism has an anabolic directivity due to synthesis of globulin proteins. At the same time, there are the signs of inhibition of albumin-synthesizing activity in liver cells (albumin in the blood decreases by 9.92%) and their "cytolytic reaction" (AlAT activity increases by 1.36 times ($p < 0.05$) and exceeds the normal range). In the blood lipidogram of the piglets, the amount of LDL-cholesterol increases by 1.44 times ($p < 0.05$) and level of triglycerides decreases by 2.64 times ($p < 0.05$), thus determining the probability of developing cardiovascular damage. The method of X-ray spectral analysis revealed the associativity of post-vaccination immunity formation with two factors: PC1 had a predominant association with indicators of natural resistance, and PC2 was associated with metabolic indicators.

The obtained results show that in order to increase the efficiency of the formation of post-vaccination immunity in the body of piglets, it is necessary to combine vaccination with hepatoprotective drugs. However, this assumption still requires experimental verification.

REFERENCES

1. Yirga, A., Jemberu, W.T., Lyons, N., Gebru, A., Akililu, F., Rushton, J. (2020). Post-vaccination herd immunity against peste des petits ruminants and inter-vaccination population turnover in small ruminant flocks in northwest Ethiopia. *Preventive Veterinary Medicin,* 174, Article 104850. https://doi.org/10.1016/j.prevetmed.2019.104850
2. Li, C., Culhane, M.R., Cheeran, M., Galina Pantoja, L., Jansen, M., Amodie, D. et al. (2020). Exploring heterologous prime-boost vaccination approaches to enhance influenza control in pigs. *Veterinary Research*, 51(1), Article 89. https://doi.org/10.1186/s13567–020–00810-z
3. Yang, S., Park, S. -J., Oh, T., Cho, H., Chae, C. (2020). Efficacy comparison of commercial porcine circovirus type 2 (Pcv2) and mycoplasma hyopneumoniae monovalent and bivalent vaccines against a dual challenge. *Canadian Journal of Veterinary Research,* 84(4), 272–282.
4. Oh, T., Park, K.H., Yang, S., Jeong, J., Kang, I., Park, C. et al. (2019). Evaluation of the efficacy of a trivalent vaccine mixture against a triple challenge with Mycoplasma hyopneumoniae, PCV2, and PRRSV and the efficacy comparison of the respective monovalent vaccines against a single challenge. *BMC Veterinary Research*, 15(1), Article 342. https://doi.org/10.1186/s12917–019–2091–6
5. Karuppannan, A.K., Opriessnig, T. (2017). Porcine circovirus Type 2 (PCV2) vaccines in the context of current molecular epidemiology. *Viruses*, 9(5), Article 99. https://doi.org/10.3390/v9050099
6. Alarcon, P, Rushton, J, Wieland, B. (2013). Cost of post-weaning multi-systemic wasting syndrome and porcine circovirus type-2 subclinical infection in England — an economic disease model. *Preventive Veterinary Medicine*, 110(2), 88–102. https://doi.org/10.1016/j.prevetmed.2013.02.010
7. Feng, H., Segalés, J., Wang, F., Jin, Q., Wang, A., Zhang, G. et al. (2022). Comprehensive analysis of codon usage patterns in Chinese porcine circoviruses based on their major protein-coding sequences. *Viruses,* 14(1), Article 81. https://doi.org/10.3390/v14010081
8. Burkov, P.V., Shcherbakov, P.N., Derkho, M.A., Rebezov, M.B. (2022). Peculiarities of post-vaccination immunity formation against porcine circovirus infection and its correction. *Agrarian Science,* 363(10), 30–36. https://doi.org/10.32634/0869–8155–2022–363–10–30–36 (In Russian)
9. Raev, S., Yuzhakov, A., Aliper, T. (2021). Whole-genome analysis of porcine circovirus type 2 in Russia. *Pathogens*, 10(12), Article 1631. https://doi.org/10.3390/pathogens10121631
10. Opriessnig, T., Gomes-Neto, J.C., Hemann, M., Shen, H.-G., Beach, N. M., Huang, Y. et al. (2011). An experimental live chimeric porcine circovirus 1–2a vaccine decreases porcine circovirus 2b viremia when administered intramuscularly or orally in a porcine circovirus 2b and porcine reproductive and respiratory syndrome virus dual-challenge model. *Microbiology and Immunology*, 55(12), 863–873. https://doi.org/10.1111/j.1348–0421.2011.00385.x
11. Lee, S.-I., Jeong, C.-G., ul Salam Mattoo, S., Nazki, S., Prasad Aganja, R., Kim, S.-C. (2021). Protective immunity induced by concurrent intradermal injection of porcine circovirus type 2 and Mycoplasma hyopneumoniae inactivated vaccines in pigs. *Vaccine*, 39(45), 6691–6699. https://doi.org/10.1016/j.vaccine.2021.07.043
12. Temple, D., Jiménez, M., Escribano, D., Martín-Valls, G., Díaz, I., Manteca, X. (2020). Welfare benefits of intradermal vaccination of piglets. *Animals,* 10(10), Article 1898. https://doi.org/10.3390/ani10101898
13. Niu, X., Ding, Y., Chen, S., Gooneratne, R., Ju, X. (2022). Effect of immune stress on growth performance and immune functions of livestock: Mechanisms and prevention. *Animals,* 12(7), Article 909. https://doi.org/10.3390/ani12070909
14. Sun, Y.I., Pence, B.D., Wang, S.S., Woods, J.A. (2019). Effects of exercise on stress-induced attenuation of vaccination responses in mice. *Medicine and Science in Sports and Exercise,* 51(8), 1635–1641. https://doi.org/10.1249/MSS.0000000000001971
15. Li, X., Liu, S., Wang, J., Yi, J., Yuan, Z., Wu, J. et al. (2021). Effects of ND vaccination combined LPS on growth performance, antioxidant performance and lipid metabolism of broiler. *Research in Veterinary Science*, 135, 317–323. https://doi.org/10.1016/j.rvsc.2020.10.007
16. Janmohammadi, A., Sheikhi, N., Nazarpak, H.H., Brujeni, G.N. (2020). Effects of vaccination on acute-phase protein response in broiler chicken. *PLoS One*, 15(2), Article e0229009. https://doi.org/10.1371/journal.pone.0229009
17. Hernández-Caravaca, I., Gourgues, S.F., Rodríguez, V., Estrada, E. D., Cerón, J.J., Escribano, D. (2017). Serum acute phase response induced by different vaccination protocols against circovirus type 2 and Mycoplasma hyopneumoniae in piglets. *Research in Veterinary Science,* 114, 69–73. https://doi.org/10.1016/j.rvsc.2017.03.006
18. Jeong, J., Kang, I., Kim, S., Park, K. H., Park, C., Chae, C. (2018). Comparison of 3 vaccination strategies against porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae,* and porcine circovirus type 2 on a 3 pathogen challenge model. *Canadian Journal of Veterinary Research*, 82(1), Article 9, 39–47.
19. Derkho, M.A., Baltabekova, A. Zh., Balabaev, B.K., Derkho, A.O. (2021). Biochemical blood profile of the Kazakh white-headed breed depending on age. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies*, 12(12), Article 12A12K, 1–11. http://doi.org/10.14456/ITJEMAST.2021.242
20. Derkho, M.A., Balabaev, B.K., Baltabekova, A. Zh., Sereda, T.I., Eliseenkova, M.V. (2022). Assessment of the influence of age and lactation period on the variability of blood biochemical composition of Kazakh whitehead cows. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies*, 13(3), Article 13A3F, 1–12. https://doi.org/10.14456/ITJEMAST.2022.48
21. Vincent, A., Awada, L., Brown, I., Chen, H., Claes, F., Dauphin, G. et al. (2014). Review of influenza A virus in swine worldwide: A call for increased surveillance and research. *Zoonoses and Public Health,* 61(1), 4–17. https://doi.org/10.1111/zph.12049
22. Jackson, D.A. (1993). Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches. *Ecology*, 74(8), 2204–2214. https://doi.org/10.2307/1939574
23. Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001). Past: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4(1), Article 4, 1–9.

24. Martelli, P., Saleri, R., Ferrarini, G., De Angelis, E., Cavalli, V., Benetti, M. et al. (2016). Impact of maternally derived immunity on piglets' immune response and protection against porcine circovirus type 2 (PCV2) after vaccination against PCV2 at different age. *BMC Veterinary Research*, 12(1), Article 77. https://doi.org/10.1186/s12917–016–0700–1
25. Sorokina, S.A., Derkho, M.A., Gizatullina, F.G., Sereda T. I. (2022). Leukocytes as indicators of the accumulation of metals in the body of growing chicks. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies*,13(4), Article 13A4R, 1–11. https://doi.org/10.14456/ITJEMAST.2022.82
26. Yanich, T.V., Derkho, M.A., Tegza, A. (2022). Hemostatic profile of holstein heifers depending on age. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies,* 13(1), Article 13A1U, 1–11. https://doi.org/10.14456/ITJEMAST.2022.21
27. Opriessnig, T., Castro, A.M.M.G., Karuppanan, A.K., Gauger, P. C., Halbur, P. G., Matzinger, S.R. et al. (2019). A porcine circovirus type 2b (PCV2b)-based experimental vaccine is effective in the PCV2b-Mycoplasma hyopneumoniae coinfection pig model. *Vaccine,* 37(44), 6688–6695. https://doi.org/10.1016/j.vaccine.2019.09.029
28. Tassis, P.D., Tsakmakidis, I., Papatsiros, V.G., Koulialis, D., Nell, T., Brellou, G. et al. (2017). A randomized controlled study on the efficacy of a novel combination vaccine against enzootic pneumonia (Mycoplasma hyopneumoniae) and porcine Circovirus type 2 (PCV2) in the presence of strong maternally derived PCV2 immunity in pigs. *BMC Veterinary Research,* 13(1), Article 91. https://doi.org/10.1186/s12917–017–1014–7
29. Rakibuzzaman, A., Piñeyro, P., Pillatzki, A., Ramamoorthy, S. (2021). Harnessing the genetic plasticity of porcine circovirus type 2 to target suicidal replication. *Viruses,* 13(9), Article1676. https://doi.org/10.3390/v13091676
30. Pooley, H.B., Begg, D.J., Plain, K.M., Whittington, R.J., Purdie, A.C., De Silva, K. (2019). The humoral immune response is essential for successful vaccine protection against paratuberculosis in sheep. *BMC Veterinary Research*, 15(1), Article 223. https://doi.org/10.1186/s12917–019–1972-z
31. Li, F., Wang, X., Ma, R., Wu, W., Teng, F., Cheng, X. et al. (2021). Oral immunization with *lactobacillus casei* expressing the porcine circovirus type 2 cap and LTB induces mucosal and xystemic antibody responses in mice. *Viruses,* 13(7), Article 1302. https://doi.org/10.3390/v13071302
32. Sobolev, O., Binda, E., O'Farrell, S., Lorenc, A., Pradines, J., Huang, Y. et al. (2016). Adjuvanted influenza-H1N1 vaccination reveals lymphoid signatures of age-dependent early responses and of clinical adverse events. *Nature Immunology*, 17(2), 204–213. https://doi.org/10.1038/ni.3328
33. Efe, C., Harputluoğlu, M., Soylu, N.K., Yilmaz, S. (2022). Letter to the editor: Liver transplantation following severe acute respiratory syndrome-coronavirus-2 vaccination-induced liver failure. *Hepatology,* 75(6), 1669–1671. https://doi.org/10.1002/hep.32409
34. Jiang, H., Wang, D., Wang, J., Zhu, S., She, R., Ren, X. et al. (2019). Induction of porcine dermatitis and nephropathy syndrome in piglets by infection with porcine circovirus type 3. *Journal of Virology,* 93(4), Article e02045–18. https://doi.org/10.1128/JVI.02045–18
35. Prajapati, K.D., Sharma, S.S., Roy, N. (2011). Current perspectives on potential role of albumin in neuroprotection. *Reviews in the Neurosciences*, 22(3), 355–363. https://doi.org/10.1515/RNS.2011.028
36. Autschbach, F., Meuer, S.C., Moebius, U., Manns, M., Hess, G., Zum Büschenfelde, K.-H. M. et al. (1991). Hepatocellular expression of lymphocyte function-associated antigen 3 in chronic hepatitis. *Hepatology,* 14(2), 223–30.
37. Sirisereewan, C., Thanawongnuwech, R., Kedkovid, R. (2022). Current understanding of the pathogenesis of porcine circovirus 3. *Pathogens,* 11(1), Article 64. https://doi.org/10.3390/pathogens11010064
38. Fukami, H., Morinaga, J., Nakagami, H., Hayashi, H., Okadome, Y., Matsunaga, E. et al. (2021). Vaccine targeting ANGPTL3 ameliorates dyslipidemia and associated diseases in mouse models of obese dyslipidemia and familial hypercholesterolemia. *Cell Reports Medicine*, 2(11), Article 100446. https://doi.org/10.1016/j.xcrm.2021.100446
39. Vania, L., Widyananda, M.H., Kharisma, V.D., Muhammad Ansori, A.N., Naw, S.W., Maksimiuk, N. et al. (2021). Anticancer activity prediction of Garcinia mangostana L. against HER2-positive breast cancer through inhibiting EGFR, HER2 and IGF1R protein: A bioinformatics study. *Biochemical and Cellular Archives*, 21(2), 3313–3321.

AUTHOR INFORMATION

Marina A. Derkho, Doctor of Biological Sciences, Professor, Institute of Veterinary Medicine, South Ural State Agrarian University. 13, Gagarin str., Troitsk, 457103, Russia. Tel.: +7–351–632–00–10, E-mail: kaf.himec@sursau.ru
https://orcid.org/0000–0003–3818–0556

Pavel V. Burkov, Candidate of Veterinary Sciences, Docent, Institute of Veterinary Medicine, South Ural State Agrarian University. 13, Gagarin str., Troitsk, 457103, Russia. Tel.: +7–351–632–00–10, E-mail: burkovpv@sursau.ru
https://orcid.org/0000–0001–7515–5670

Pavel N. Shcherbakov, Doctor of Veterinary Sciences, Docent, Institute of Veterinary Medicine, South Ural State Agrarian University. 13, Gagarin str., Troitsk, 457103, Russia. Tel.: +7–351–632–00–10, E-mail: scherbakov_pavel@mail.ru
https://orcid.org/0000–0001–8685–4645

Maksim B. Rebezov, Doctor of Agricultural Sciences, Candidate of Veterinary Sciences, Professor, Leading Researcher, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia. Tel.: +7–999–900–23–65, E-mail: rebezov@yandex.ru
https://orcid.org/0000–0003–0857–5143
* corresponding author

Ksenia V. Stepanova, Candidate of Biological Sciences, Docent, Institute of Veterinary Medicine, South Ural State Agrarian University. 13, Gagarin str., Troitsk, 457103, Russia. Tel.: +7–351–632–00–10, E-mail: deratizator@bk.ru
https://orcid.org/0000–0002–3916–004X

Arif N. M. Ansori, Doctor in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kec. Mulyorejo, Surabaya, East Java, 60115, Indonesia. E-mail: arif.nma-17@fkh.unair.ac.id
https://orcid.org/0000–0002–1279–3904

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.