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Correction of cellular and humoral links immunity in piglets under the weaning condition

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Abstract. Preventing the development of immunodeficiencies in piglets after weaning is achievable through the parenteral administration of fat-soluble vitamins, macro- and microelements, and by analysing their effects on the dynamics of changes in the number and functional activity of T- and B-lymphocyte subpopulations. Accordingly, this study aimed to investigate the effect of a complex liposomal preparation on the cellular and humoral components of the piglet immune system after weaning. A standardised methodology was used to determine the number of different populations

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and subpopulations of T-lymphocytes in piglet blood samples collected before and at specific intervals after weaning. The analysis of the quantitative composition of T- and B-lymphocytes and the functional activity of T lymphocytes in the blast transformation reaction was conducted using immersion microscopy of smears. The statistical processing of results was carried out through variational nonparametric analysis using biometric methods. The findings revealed that weaning from sows caused a reduction in the number of T-cells of varying degrees of avidity, a decrease in the relative number of T-suppressors in piglets' blood, and an increase in the number of certain subpopulations of T-helper cells. Conversely, the administration of the liposomal preparation to piglets enhanced the number of various subpopulations of total and active T-lymphocytes and B-lymphocytes in their blood after weaning and increased the activity of lymphoid cells in the blast transformation reaction with phytohaemagglutinin. The observed immunomodulatory effect of the tested drug is attributed to the synergistic combination of fat-soluble vitamins, mineral elements, and arginine in its composition, which effectively prevented the development of stress-induced immunodeficiency in piglets following weaning

Keywords: immunodeficiencies; liposomal preparation; lymphocytes populations; weaning; stress; arginine

INTRODUCTION

Pigs are highly suitable subjects for studying the effect of stress on the body as a factor contributing to immunodeficiency. It is well established that animals of this species exhibit exceptionally high sensitivity to stress factors. Due to the demands of modern production systems, technological stress affects pigs throughout their entire development. One of the unavoidable stressors in the life of piglets is weaning from sows. Weaning is accompanied by activation of the sympathoadrenal and hypothalamic-adenohypophysis-adrenocortical systems and disruption of the pro-oxidant-oxidant balance in the piglets' bodies. This imbalance reduces natural resistance and immune reactivity during weaning, serving as a key factor in developing acute and chronic diseases in piglets (Jia *et al.,* 2020; Hao *et al.,* 2021). According to A. Luppi *et al.* (2023), weaned piglets are predisposed to diseases of both infectious and non-infectious aetiology, with toxic liver damage (hepatosis and hepatitis), gastroenteritis, and respiratory diseases (bronchitis and bronchopneumonia) being the most common.

Moreover, B. Jahangiri *et al.* (2022) demonstrated a functional relationship between these diseases. For instance, in respiratory diseases, liver activity is suppressed due to tissue hypoxia, inhibition of aerobic processes, intensification of anaerobic reactions, and the development of acidosis in the liver parenchyma. These processes lead to dystrophic and necrotic changes, reducing the liver's synthetic and detoxification functions. Chronic hypoxia also causes functional insufficiency in haematopoietic and immunoregulatory cells, leading to secondary immunodeficiencies. B. Foglia *et al.* (2021) highlighted that non-specific gastroenteritis in weaned piglets is associated with hyperchromic anaemia and hyperenzymopathy, along with a significant reduction in the De Ritis ratio, indicative of parenchymal liver damage and structural changes in the pancreas. Similarly, as shown by C. Lauridsen *et al.,* (2021), the transition from a milk-based diet to concentrated feed reduces lipid assimilation in piglets, leading to insufficient digestion of nutrients and resulting in dystrophic processes in the liver.

The above discussion highlights the need to stimulate piglet immunity to prevent potential disorders caused by weaning from sows. Modern methods of regulating immunogenesis involve the use of medicines that target immunocompetent cells, enhance their functional properties, and activate interactions with antigenic material. In this context, fat-soluble vitamins – particularly retinol, tocopherol, and calciferol – along with arginine and several macro- and microelements, are noted for their powerful protective effects on the body (Rufino-Moya *et al.*, 2020; Buchko *et al.,* 2024). M. Khariv *et al.* (2017) observed that liposomal preparations, which remain in the body for extended periods, can serve as a significant alternative to conventional medicinal forms. Encapsulation of active substances, particularly hydrophobic ones, in liposomes, increases their bioavailability and allows modification of their release. Under certain conditions, liposomes are absorbed by cells and merge with cell membranes, enabling the direct transport of their contents into the cell (Nsairat *et al.,* 2022). However, despite their high efficacy, liposomal preparations are rarely used in veterinary medicine. In this context, studying the state of the cellular and humoral components of piglet immunity during weaning and developing effective complex preparations in the form of liposomal emulsions for its regulation is a pressing area of scientific research.

Thus, the experiments aimed to develop and evaluate the effects of an improved dosage form of an immunotropic preparation – based on fat-soluble vitamins, macro- and microelements – on the bodies of piglets during weaning from sows.

MATERIALS AND METHODS

The experiment was conducted at the "Flora-S" enterprise in the Sokal District of the Lviv Region, using two groups of Large White breed piglets, each comprising nine animals. Two days prior to weaning, the control

group of piglets was parenterally injected with 0.1 mL of NaCl, while the experimental group received an equivalent amount of liposomal preparation. The composition of 1 L of this preparation included the following: vitamin A – 900-1100 IU, vitamin D3 – 1100-1300 IU, vitamin E – 0.9-1.1 mg, arginine – 1.8-2.2 mg, zinc acetate – 0.9-1.1 mg, sodium selenite – 0.090.11 mg, cobalt acetate – 0.2-0.4 mg, and magnesium sulphate – 0.9-1.1 mg. During the study, all animals were fed "Prestarter for Piglets TN" (Trouw Nutrition, Belgium), a combined feed designed for piglets aged 5-65 days.

Blood samples from piglets were collected from the cranial vena cava two days prior to weaning and on the 1st, 5th, and 10th days following weaning. Lymphocytes were isolated from the blood using a ficoll-verographin gradient with a relative density of 1.077. The ficoll-verographin gradient was prepared by adding a 9% solution of Ficoll (Pharmacia, Sweden) to a 34% solution of Verographin (Spofa, Czech Republic). Lymphocytes were incubated in Basal Medium Eagle (Sigma, USA) with ram erythrocytes. During incubation, erythrocytes adhered to T-cells, forming rosette-like structures. B-lymphocytes were identified based on their membrane receptors for C3b-C3d complement components and the Fc fragment of immunoglobulin. Ram erythrocytes treated with trypsin (Biopharma, Ukraine) were used as indicator cells. Lymphocytes transformed into blasts through activation by phytohaemagglutinin (Lectynotest, Ukraine) from their inactive forms. Cytological preparations were fixed in methanol (ChemStore, Netherlands), stained using Romanowsky-Giemsa (RG) stain, and observed under a binocular microscope (J-CM2000B, Zenith Lab, China).

The number of T-lymphocytes in the blood of piglets was counted according to a modified and improved method (Vishchur *et al.,* 2007). Active rosette-forming lymphocytes, with receptors capable of attaching sheep erythrocytes without incubation, were isolated, and the number of helper lymphocytes forming rosettes after incubation with theophylline was also determined. To determine B-lymphocytes, the EAS system (erythrocytes sensitised with antibodies and complement) was prepared by adding haemolytic serum to sheep erythrocytes. Rosettes were counted using smear microscopy under immersion. Based on the number of sheep erythrocytes attached, lymphocytes were classified as follows: undifferentiated (zero) – not attaching a single erythrocyte; low-avidity – attaching 3-5 erythrocytes; medium-avidity – attaching 6-10 erythrocytes; and high-avidity (morula) – attaching more than 10 erythrocytes.

T-suppressors were determined by calculating the difference between the number of helper Tlymphocytes and the total number of T-lymphocytes. The immunoregulatory index (IRI) was calculated based on the ratio of T-helpers to suppressors. The functional activity of T cells was assessed using the lymphocyte blast transformation (LBT) reaction, with phytohaemagglutinin used as a mitogen (Vlizlo *et al.,* 2012). The percentage of blasts was calculated relative to the total number of T cells. The data were analysed using the Statistica 7.0 software package (StatSoft Inc., USA). Results were statistically evaluated by calculating the arithmetic mean ($\bar{x}$) and its standard deviation (SD) using ANOVA. The significance of mean differences was assessed with Student's t-test. All interventions complied with the international principles outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), ISO/IEC 17025:2005 (2006), and the Law of Ukraine No. 249 (2012). Ethical approval for the study was obtained according to Protocol No. 93 (03.06.2021) from the Bioethical Commission of the Institute of Animal Biology of NAAS.

RESULTS

According to the obtained data, weaning from sows exerts an inhibitory effect on blood lymphocytes, particularly on the total populations in the control piglets (Table 1). This effect was most pronounced in the first days following weaning. For instance, on the 1st day after weaning, compared to the period before weaning, a 1.14-fold reduction in low-avidity T-total lymphocytes and a twofold reduction in high-avidity T-total lymphocytes were observed in the blood of piglets in the control group. By the 5th day, a 1.93-fold decrease in medium-avidity forms was recorded.

Table 1. *Total T-lymphocytes and their functional activity in the piglets' blood (x ± SD; %; n = 9)*

Indicators	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
T-general, attached 0 erythrocytes (undifferentiated cells)	C	52.01 ± 2.32	54.62 ± 2.91	55.69 ± 2.40	55.05 ± 2.31
	E		51.67 ± 3.52	53.30 ± 2.93	57.10 ± 2.34
attached 3-5 erythrocytes (low avidity)	C	37.14 ± 1.19	32.34 ± 0.65^{00}	36.57 ± 1.76	35.23 ± 3.06
	E		35.32 ± 1.24*	36.08 ± 1.17	32.03 ± 1.22
attached 6-10 erythrocytes (medium avidity)	C	9.08 ± 1.21	11.23 ± 0.66^{0}	4.62 ± 0.69^{00}	7.37 ± 1.34
	E		10.01 ± 1.16	7.12 ± 1.18*	7.14 ± 1.17

Table 1. Continued

Indicators	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
Morulas (high avidity)	C	3.22 ± 0.22	1.50 ± 1.01⁰	3.00 ± 1.18	2.49 ± 0.64
	E		3.03 ± 1.18	3.67 ± 2.39	4.22 ± 1.19
% relative amount	C	48.31 ± 2.35ᵃ	45.38 ± 2.85	44.32 ± 2.42	45.31 ± 2.32
	E		48.31 ± 3.47	46.59 ± 2.95	43.18 ± 2.29

Note: *C – control, E – experimental. * – probable differences compared to the control: * – p < 0.05; ** – p < 0.01; *** – p < 0.001; 0 – in relation to the period before weaning: 0 – p < 0.05; 00 – p < 0.01; 000 – p < 0.001; 0 – undifferentiated cells*
Source: *developed by the authors*

For the experimental group of piglets, compared to the control group, a 1.09-fold higher number of low-avidity T-lymphocytes was noted in the blood on the 1st day after weaning. Additionally, the number of medium-avidity T-total lymphocytes increased by 1.49 times on the 5th day post-weaning. In terms of high-avidity forms and the relative number of total lymphocytes, the experimental group consistently demonstrated a tendency towards increased values compared to the control across nearly all post-weaning periods of the study.

On the 1st and 10th days after weaning from sows, a tendency to a reduction in the number of functionally undifferentiated T-active lymphocytes was found in the blood of piglets in the control group. However, on the 5th day post-weaning, a decline in the number of low-avidity cells was observed (Table 2). The relative number of active T-lymphocytes in the blood of the control group piglets, compared to the pre-weaning period, increased at all stages post-weaning due to an elevation in the number of medium-avidity forms. In contrast, an increase in the number of low-avidity forms of active T-lymphocytes was observed at all stages of research in the experimental group, along with a rise in medium-avidity cells by the 10th day after weaning.

Table 2. *Active T-lymphocytes and their functional activity in the piglets' blood (x ± SD; %; n = 9)*

Indicator	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
T-active, attached 0 erythrocytes (undifferentiated cells)	C	81.19 ± 2.32	78.59 ± 3.52	81.22 ± 2.03	78.70 ± 0.62
	E		79.61 ± 3.48	78.13 ± 2.42	76.37 ± 3.51
attached 3-5 erythrocytes (low avidity)	C	18.06 ± 1.20	18.48 ± 1.76	17.12 ± 3.07	18.03 ± 2.05
	E		19.11 ± 3.06	20.07 ± 1.18	18.31 ± 1.78
attached 6-10 erythrocytes (medium avidity)	C	1.52 ± 1.03	2.72 ± 1.81	2.31 ± 0.71	3.04 ± 1.14
	E		1.35 ± 0.68	1.69 ± 1.34	5.32 ± 1.75
% relative amount	C	19.04 ± 2.29	21.41 ± 3.46	19.35 ± 0.69	21.05 ± 2.30
	E		20.29 ± 3.54	21.68 ± 2.45	23.66 ± 3.52

Note: *C – control, E – experimental. * – probable differences compared to the control: * – p < 0.05; ** – p < 0.01; *** – p < 0.001; 0 – in relation to the period before weaning: 0 – p < 0.05; 00 – p < 0.01; 000 – p < 0.001; 0 – undifferentiated cells*
Source: *developed by the authors*

In the blood of piglets from the control group at the final stage of research after weaning, an increase in the relative number of T-helpers and their medium-avidity forms was observed against the backdrop of a decrease of undifferentiated cells (Table 3). Conversely, the relative proportion of T-suppressors in the control group decreased after weaning compared to the pre-weaning period. In comparison to the control group, the number of low-avidity T-helper forms in the experimental group decreased by 1.31 times at the beginning of the study, while T-suppressors demonstrated a similar reduction at the end of the research.

Table 3*. The number of T-helpers and T-suppressors, their functional activity, immunoregulatory index, and lymphocyte blast transformation in the piglets' blood (x ± SD; %; n = 9)*

Indicators	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
T-helpers, attached 0 erythrocytes (undifferentiated cells)	C	80.12 ± 2.29	76.98 ± 2.35	77.31 ± 4.80	75.94 ± 1.18⁰
	E		79.65 ± 3.51	74.08 ± 3.46	73.02 ± 3.46

Table 3. Continued

Indicators	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
attached 3-5 erythrocytes (low avidity)	C	20.23 ± 2.31	21.89 ± 1.17	18.65 ± 3.05	20.07 ± 2.33
	E		16.58 ± 0.88**	21.34 ± 1.77	22.99 ± 1.14
attached 6-10 erythrocytes (medium avidity)	C	1.57 ± 1.04	1.52 ± 1.02	4.02 ± 2.30	4.01 ± 1.21⁰
	E		3.64 ± 1.76	4.68 ± 1.80	4.05 ± 1.29
% relative amount	C	20.04 ± 2.45	23.05 ± 2.29	22.67 ± 4.82	23.89 ± 1.19⁰
	E		20.32 ± 3.46	25.96 ± 3.47	26.95 ± 3.46
T-suppressors, %	C	27.99 ± 0.21	22.37 ± 0.53⁰⁰⁰	21.68 ± 2.39⁰⁰	21.11 ± 3.02⁰
	E		27.94 ± 6.12	20.62 ± 5.34	16.03 ± 0.58*
Immunoregulatory index	C	0.71 ± 0.08	1.03 ± 0.07⁰⁰	1.06 ± 0.32	1.16 ± 0.11⁰
	E		0.76 ± 0.31	1.31 ± 0.45	1.70 ± 0.19*
Lymphocyte blast transformation	C	44.97 ± 2.13	39.97 ± 1.14⁰	44.33 ± 2.41	41.33 ± 2.41
	E		41.62 ± 0.59	46.66 ± 3.52	46.71 ± 1.76*

Note: *C – control, E – experimental. * – probable differences compared to the control: * – p < 0.05; ** – p < 0.01; *** – p < 0.001; 0 – in relation to the period before weaning: 0 – p < 0.05; 00 – p < 0.01; 000 – p < 0.001; 0 – undifferentiated cells*
Source: *developed by the authors*

The immunoregulatory index increased in the control group throughout all periods following weaning compared to the pre-weaning period. At the same time, for piglets in the experimental group, the value of the immunoregulatory index exceeded the control value by 1.46 times on the 10th day after weaning. The more pronounced increase in the immunoregulatory index in the experimental group, compared to the control, during the specified periods post-weaning can be attributed to an increase in the relative number of T-active lymphocytes and T-helpers, along with a decrease in the number of T-suppressors. Apparently, the stress experienced by piglets during weaning from sows results in a weakening of the ability of lymphocytes to transform into blasts, as evidenced by a significant 1.13-fold reduction in lymphocyte blast transformation in the blood of the control group on the day following weaning. Administration of the liposomal preparation to piglets led to increased lymphocyte blast transformation in the blood compared to the control group, with the effect being particularly pronounced at the end of the study.

The results shown in Table 4 indicate that the weaning of piglets from sows does not significantly affect the number and functional activity of B-lymphocytes but only leads to a tendency for their low-avidity and high-avidity forms to decrease in the blood on the 1st day after weaning. Conversely, for piglets in the experimental group, the relative number of B-lymphocytes exhibited a tendency to increase throughout all post-weaning periods compared to the control group. In general, compared to the control, the number of low-avidity forms in the blood of piglets in the experimental group increased by 1.11 times at the end of the study, while the number of medium-avidity forms of this lymphocyte population rose by 1.55 times on the 5th day after weaning.

Table 4. *The number of B-lymphocytes and their functional activity in the piglets' blood (x ± SD, %, n = 9)*

Indicators	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
B-lymphocytes, attached 0 erythrocytes (undifferentiated cells)	C	54.93 ± 2.31	54.67 ± 4.06	55.96 ± 2.32	53.64 ± 1.77
	E		51.07 ± 3.45	52.88 ± 3.47	50.06 ± 2.73
attached 3–5 erythrocytes (low avidity)	C	28.76 ± 1.17	26.11 ± 2.25	26.69 ± 2.41	29.95 ± 1.18
	E		26.38 ± 2.42	22.93 ±1.19	31.02 ± 1.15*
attached 6–10 erythrocytes (medium avidity)	C	9.02 ± 1.12	13.96 ± 2.03	10.35 ± 0.64	10.13 ± 1.38
	E		16.64 ± 2.91	16.07 ± 0.98***	11.20 ± 1.22
Morulas (high avidity)	C	7.11 ± 1.28	5.35 ± 1.34	6.95 ± 1.18	8.35 ± 0.69
	E		6.02 ± 1.18	8.07 ± 1.21	8.09 ± 1.58
% relative amount	C	44.89 ± 2.40	45.34 ± 4.08	43.91 ± 2.35	46.30 ± 1.76
	E		49.08 ± 3.46	47.12 ± 3.39	50.08 ± 3.40

Note: *C – control, E – experimental. * – probable differences compared to the control: * – p < 0.05; ** – p < 0.01; *** – p < 0.001; 0 – in relation to the period before weaning: 0 – p < 0.05; 00 – p < 0.01; 000 – p < 0.001; 0 – undifferentiated cells*
Source: *developed by the authors*

The obtained data indicate that weaning of piglets causes them to experience a stressful state, leading to a decrease in the number of total T-lymphocytes of varying avidity, a reduction in the relative number of T-suppressors, and an increase in certain subpopulations of T-helpers in their blood. In contrast, the liposomal preparation demonstrated a stimulatory effect on the functional activity of immune cells. This preparation had a normalising effect on the number of various subpopulations of T- and B-lymphocytes in the blood of piglets during different stages of weaning. In particular, parenteral administration of a liposomal preparation containing vitamins A, D3, and E, along with arginine, zinc acetate, sodium selenite, cobalt acetate, and magnesium sulphate, contributed to an increase in the number of total and active T-lymphocyte and B-lymphocyte subpopulations, as well as the activity of lymphoid cells in the reaction of lymphocyte blast transformation with phytohaemagglutinin.

DISCUSSION

There are two main forms of specific immune response: cellular and humoral. T-lymphocytes constitute the cell-mediated component of the immune system, recognising antigens through specific receptors located on their surface (Ohorodnyk *et al.*, 2017). Lymphocytes are cells that perform not only the specific functions of immune protection but also act as elements of a unified information system. This system accurately reflects the state of the body at a given time or under the influence of certain factors and enables the evaluation of the therapeutic effects of studied preparations (Cerda *et al.*, 2022). The obtained data indicate that weaning from sows exerts an inhibitory effect on the total population of T-lymphocytes in piglets' blood. This effect is evident from the first day after weaning, with a significant decrease observed in the number of low-avidity ($p < 0.05$) and highavidity ($p < 0.05$) forms of T-total lymphocytes. On the 5th day, this is further evidenced by a reduction in the number of their medium-avidity forms ($p < 0.05$). The post-weaning decline in the relative number of total T-lymphocytes in the blood of piglets from the control group is attributed to the action of prostaglandins and interleukin-2 (IL-2). This is because the inhibition of the T-cell response to an antigen occurs during the IL-2-dependent stimulation of T-lymphocytes (Lykhopiy *et al.*, 2023). Under stress, the activation of receptors for this cytokine on the surface of T-lymphocytes is reduced (Niederlova *et al.,* 2023; Raeber *et al.,* 2023). It appears that weaning from sows also affects the transcription factor NF-kB, whose d activity leads to T-lymphocyte dysfunction, a reduction in their proteolytic activity, and the emergence of signs of immunodeficiency (ParraLlorca *et al.,* 2023).

In the blood of piglets in the experimental group on the first day after weaning, an increase in the number of low-avidity forms of lymphocytes ($p < 0.05$) was observed, as well as an increase in the number of medium-avidity forms of T-total lymphocytes on the 5th day. During nearly all periods of observation following the weaning of piglets, there was a consistent tendency towards an increase in high-avidity forms and the relative number of total lymphocytes. It should be noted that the increase in the number of high-avidity forms and the relative number of total lymphocytes in the blood of piglets after weaning, as influenced by liposomal preparation, represents a positive outcome. This is because it is known that almost all mature T-lymphocytes express CD3 marker molecules on their surface, which are involved in transmitting signals from the T-cell receptor into the cells, thereby stimulating activation and proliferation processes. The reduction in the number of T-suppressors in the blood of piglets after the administration of the liposomal preparation on the 10th day after weaning suggests that immune aggression processes were stabilising within this period. This stabilisation concludes with the interaction of T-killer receptors with target cells in the presence of CD8. Cytotoxic T-lymphocytes with the CD8 phenotype regulate antibody production and the formation of immune tolerance. They also induce the death of infected cells and act on infectious agents. Due to their immunosuppressive function, CD8 prevents autoaggression and influences the development of immune responses, directing them towards either a humoral or cellular response (VieyraLobato *et al.,* 2018; Xie *et al.,* 2021).

The implementation of the humoral link of immunity in the body is carried out by Blymphocytes, which, under the influence of an antigenic stimulus, differentiate into plasma cells that produce antibodies (Rastogi *et al.,* 2022). It is evident that stress factors inhibit antibody formation processes, while the production of lymphocyte-activating factors by macrophages is induced, and corticosteroids are released. These corticosteroids bind to specific lymphocyte receptorsforming, complexes that disrupt the production of lymphokines. Simultaneously, due to a reduction in cooperation between T- and B-lymphocytes, T-dependent antibody production is inhibited, and the differentiation of B-lymphocytes as well as the synthesis of IgM and IgG are impaired (Rodriguez-Mogeda *et al.,* 2024). Under the conditions of this study, the weaning of piglets from sows does not significantly affect the number and functional activity of B-lymphocytes but only shows a tendency towards a decrease in low-avidity and high-avidity forms in their blood on the 1st day after weaning. Meanwhile, in piglets of the experimental group, the relative number of Blymphocytes showed a tendency to increase throughout all post-weaning periods.

The observed changes in the functional state of the receptor apparatus of T- and B-lymphocytes in the blood of piglets in the experimental group suggest that the combined application of all components of the liposomal preparation is an effective means for preventing

immunodeficiencies. This preparation activates the cellular and humoral mechanisms of the immune response in their bodies after weaning from sows. To fully understand the immunomodulatory effect of the liposomal preparation on the body of piglets post-weaning, it is essential to examine in detail the various components it contains, particularly the fat-soluble vitamins, L-arginine, zinc, selenium, cobalt, and magnesium. Retinol is a crucial vitamin for the formation of the body's defence mechanisms (Liu *et al.,* 2019). By increasing the secretion of IL-2 through RAR receptors, it stimulates cellular reactions and the proliferation of T-lymphocytes, activates the humoral link of the immune response to T-dependent antigens, enhances the activity of cytotoxic lymphocytes, and reduces the synthesis of IL-10 and IL-12 (Ouyang & O'Garra, 2019).

One of the key roles in lymphocyte formation is played by calciferol, the active form of which – $1.25(OH)_2D_3$ – influences natural resistance and specific immunity, stimulates lymphocytes with concanavalin A, and induces cathelicidin and IL-1 gene expression (Ao *et al.,* 2021). Vitamin D_3 enhances phagocyte numbers, promotes interferon synthesis and T-lymphocyte activity, and stimulates enzyme systems involved in the generation of reactive oxygen species in immunocompetent cells (Sirbe *et al.,* 2022). Receptors for $1.25(OH)_2D_3$ have been identified in 5% of activated T-lymphocytes and 95% of B-lymphocytes (Cyprian *et al.,* 2019). Specific receptor proteins for $1.25(OH)_2D_3$ have been detected in immature thymus cells, with a significant proportion found in $CD8^+$ and $CD4^+$. The vitamin D_3 cells hydroxylase activity of immunocompetent cells enables them to produce hormonally active forms of vitamin D_3. Activated macrophages express receptors for vitamin D_3, monocytes and lymphocytes express a 50 kDa receptor protein, and lymphocytes express an 80 kDa cytosolic protein (Liang *et al.,* 2019). The signals from these proteins affect TGF, which regulates the differentiation and maturation of progenitors of stem cells, monocytes, macrophages, and lymphocytes capable of synthesising cytokines, growth factors, and calcium-dependent mediators of immunogenesis (Fisher *et al.,* 2019).

At the same time, the tocopherol present in the preparation stimulates the transformation of lymphocytes into blasts. Vitamins E and D_3 inhibit the activity of the nuclear factor NF-kB, which is involved in the development of immunodeficiency and the regulation of genes responsible for forming adhesion molecules (VCAM, ICAM), TNF, IL-1, IL-2, IL-6, IL-8, and γ-IFN. These vitamins prevent the dissociation of the NF-kB complex with activator protein-1 (AP-1) (Mussbacher *et al.,* 2019). This complex regulates the proliferation and interaction of cells during immune response development. Normally, it is blocked by the cellular inhibitor AP-1, but under stress conditions, it dissociates, allowing the released NF-kB to be transported into the nucleus, where genes that cause apoptosis are expressed.

Zinc restores the levels of Th1 and Th2 cells and affects IL-6, which increases the cytotoxic effect of T-lymphocytes. It also acts on the immunostimulatory cytokine IL-1 (Prasad, 2020). IL-1, in turn, influences the hypothalamus, which is responsible for coordinating neuro-endocrine-immune interactions in the body that potentiate the immune response during the inductive phase. Overall, the action of these interleukins involves enhancing B-lymphocyte differentiation, increasing antibody production, protecting cells from apoptosis, and stimulating haematopoiesis processes (Da Silva Lima *et al.,* 2023). IL-1 and IL-6 act as co-stimulators of T cells and thymocytes, increasing neutrophil chemotaxis and the release of lysozyme from cells, thus contributing to the normal phagocytosis of antigens (Rybtsova *et al.,* 2023).

The trace element selenium also exerts a stimulatory effect on lymphocyte activity in the blood of piglets in the research group after weaning from sows. This effect is attributed to its ability to mitigate the destructive impact of stress on lymphocytes (Da Silva Duarte *et al.,* 2022). In turn, the cobalt preparation included in the study in small quantities inhibits oxidation processes (Kosiorek & Wyszkowski, 2019), which consequently prevents the formation of lipid peroxidation products in piglets after weaning. Stress conditions are known to lower magnesium levels in the body; however, supplementation with magnesium has been shown to increase the synthesis of CD3, CD16, CD22, IL-1, and immunoglobulins (IgG, IgA, IgE). Magnesium also supports the differentiation of T-killers into mature effectors and reduces the formation of reactive oxygen species (Shimasaki *et al.,* 2020). According to M. Pedrazzini *et al.* (2024), arginine enhances the functional activity of lymphocytes. Moreover, it is known that in young animals and under stressful conditions, the levels of this amino acid in the body decline sharply, making it essential. Thus, the inclusion of arginine in the studied preparation likely had an immunotropic effect on the piglets' bodies, increasing their antioxidant status and reducing the intensity of free radical oxidation, thereby limiting the impact of stress. Therefore, the injection of the liposomal preparation containing vitamins A, D_3, E, arginine, zinc acetate, sodium selenite, cobalt acetate, and magnesium sulphate eliminates the stress state in piglets caused by weaning from sows.

CONCLUSIONS

The studies indicate that weaning from sows results in an increase in the number of T-helpers in the blood of piglets on the 10th day after weaning, a decrease in the number of T-suppressors throughout the studied periods, and a reduction in the transformation ability of T-lymphocytes into blasts on the 10^{th} day after weaning. In contrast, the combination of fat-soluble vitamins, arginine, zinc, selenium, cobalt, and magnesium in the liposomal preparation enhanced the immunoregulatory index, increased T-lymphocyte activity in the blast

transformation reaction, and reduced the number of T-suppressors in the blood of piglets on the 10th day after weaning from sows. The positive effect of the investigated liposomal preparation on the activity of the cellular and humoral links of the immune system in piglets is attributed to the synergistic action of vitamins, trace elements, and Larginine, which effectively mitigated the stress induced by weaning from sows. Further research is recommended to examine the effect of the immunotropic liposomal preparation on antioxidant protection markers, the nitric oxide system, and lipid and mineral metabolism in the bodies of piglets.

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CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be interpreted as a potential conflict of interest. All authors have read and approved the final manuscript.

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Анотація. Профілактика розвитку імунодефіцитів у поросят при відлученні від свиноматок можлива шляхом парентерального введення жиророзчинних вітамінів, макро- і мікроелементів та дослідження їх впливу на динаміку змін кількості й функціональної активності субпопуляцій Т- і В-лімфоцитів. Відповідно метою цих досліджень було дослідження впливу комплексного ліпосомального препарату на клітинну і гуморальну ланку імунної системи поросят після відлучення від свиноматок. Для визначення у зразках крові поросят, відібраної до та в окремі періоди після відлучення від свиноматок, кількості різних популяцій і субпопуляцій Т-лімфоцитів використовували уніфіковану методику. Аналіз кількісного складу Т- і В-лімфоцитів й дослідження функціональної активності Т-лімфоцитів у реакції бластної трансформації проводили шляхом мікроскопії мазків під імерсією. Статистичну обробку отриманих результатів виконували за допомогою варіаційного непараметричного аналізу з використанням біометричного методу. Як показали дослідження, відлучення від свиноматок спричиняє зменшення у крові поросят кількості Т загальних лімфоцитів різного ступеня авідності та відносної кількості Т-супресорів й зростання чисельності окремих субпопуляцій Т-хелперів. Натомість введення поросятам ліпосомального препарату сприяло збільшенню в їх крові у період після відлучення кількості різних субпопуляцій загальних і активних Т-лімфоцитів та В-лімфоцитів й викликало зростання активності лімфоїдних клітин у реакції бластної трансформації з фітогемаглютиніном. Отриманий імуномодулюючий вплив досліджуваного препарату зумовлений комплексним поєднанням у його складі жиророзчинних вітамінів, мінеральних елементів та аргініну, що дозволило ефективно запобігти розвитку в організмі поросят імунодефіциту, спричиненого дією стресу, внаслідок відлучення від свиноматок

Ключові слова: імунодефіцити; ліпосомальний перпарат; популяції лімфоцитів; стрес; аргінін