SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 28(2), 9-22

UDC 612.017:636.4-663.127
DOI: 10.48077/scihor2.2025.09

The state of T- and B-cell links of specific immunity and activity of humoral factors of natural defence of sows and their piglets under the influence of *Saccharomyces Cerevisiae* yeast complex and enzymes

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Article's History:
Received: 12.10.2024
Revised: 05.01.2025
Accepted: 22.01.2025

Abstract. The immaturity of the immune system of newborn piglets causes significant losses in the postnatal period and is completely dependent on the mother's body, especially in the last period of pregnancy, due to increased metabolic processes and physiological immunosuppression. The study aimed to determine the activity of natural

Suggested Citation:
Prudyus, T., Vishchur, O., Mudrak, D., Gutsol, A., & Gutsol, N. (2025). The state of T- and B-cell links of specific immunity and activity of humoral factors of natural defence of sows and their piglets under the influence of *Saccharomyces Cerevisiae* yeast complex and enzymes. *Scientific Horizons*, 28(2), 9-22. doi: 10.48077/scihor2.2025.09.

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and adaptive defence factors in sows and piglets born from them under the influence of a feed additive containing *Saccharomyces cerevisiae* yeast and enzymes. The results of the research demonstrated that feeding sows during pregnancy and lactation, as well as piglets before weaning, had a positive effect on the immune function of the mother and offspring. Under the influence of these factors, the additive has an activating effect on the natural mechanisms of the humoral link of the body's nonspecific resistance, especially in sows. This was evidenced by the higher bactericidal and lysozyme activity of sow blood serum during pregnancy and lactation ($p < 0,05\text{-}0,001$) and a lower content of circulating immune complexes ($p < 0,05$), which indicates a decrease in the antigenic load on the mother's body, especially during the period of immunosuppression. Similar changes, but expressed to a lesser extent, were recorded in piglets, with higher BASK ($p < 0.05$) and lower CIC ($p < 0.05$). Feeding sows and piglets with yeast and enzymes during periods of low immune potential of their body had an activating effect on the state of adaptive immunity, in particular, cellular factors of specific body defence. This was evidenced by an increase in the number of T-lymphocytes (total, active and theophylline-resistant) and B-lymphocytes ($p < 0.05\text{-}0.001$) and an increase in their functional activity due to the redistribution of the receptor apparatus of immunocompetent cells – an increase in the number of T- and B-lymphocytes with low and medium receptor density and a decrease in undifferentiated cells ($p < 0.05\text{-}0.001$). Thus, the activating effect of the studied factors of the feed additive on the state of natural and adaptive defence factors in the body of sows and their offspring was established, which positively affects the formation of an immune response in periods of low immune potential of their body

Keywords: piglets; *Saccharomyces cerevisiae* yeast; probiotics; T- and B-lymphocytes; immunological tests

INTRODUCTION

The safety of piglets at an early age and during weaning is one of the most pressing issues in livestock production not only in Ukraine but also in most developed countries. The immune system is a major contributor to the viability of the organism. At the same time, in the context of modern intensive livestock farming, which is unilaterally focused solely on productive animal feeding, environmental stress, several stress factors and uncontrolled use of chemotherapeutic agents lead to a violation of metabolic homeostasis, a decrease in the level of cellular and humoral defence factors, the development of inflammation and immune deficiency, which can cause diseases. At the same time, in most cases, a violation of the quantitative and qualitative composition of the microbiocinosis of the intestinal tract is recorded and, as a result, the occurrence of bacterial imbalance in piglets. A pathologically altered microbial ecosystem can be a trigger for the development of the disease, contribute to its chronicity with the development and progression of metabolic and immune disorders, and the formation of endogenous infection reservoirs of various aetiologies and localisation. Therefore, there is a need to use appropriate remedies to correct impaired functions, and to restore microbiocinosis and the body's immune potential.

It is, therefore, necessary to develop and introduce into production safe products that can take a prominent place in the system of measures to ensure the biological protection of animals, especially young piglets. Scientists such as M. Elghandour *et al.* (2020), T. Pruduys and O. Vishchur (2022) believe that probiotic supplements, which are used as growth stimulants and are an alternative to feed antibiotics, largely meet these requirements. Probiotics are live bacteria or yeast cultures that are used to stabilise digestion, inhibit the growth of pathogens, and produce vitamins, enzymes amino acids and other biologically active substances.

An analysis of periodicals and specialised literature has shown that several major US, UK and European manufacturers have begun to supply the Ukrainian market with new-generation feed additives of various types: flavour and aroma substances, enzyme preparations, probiotics, prebiotics and others. At the same time, domestic producers, using modern technologies, are also entering the market for high-quality, safe, competitive feed additives. However, the successful use of such additives requires a high-quality comprehensive study of their efficacy and safety, as well as clear dosage recommendations. In this context, various yeast strains that can be used as probiotics, prebiotics, synbiotics and postbiotics are attracting particular attention. H. Vries *et al.* (2020) believe that the use of *Saccharomices cerevisiae* yeast in monogastric animals is of particular interest. The authors state that the main effect of adding yeast is to stimulate the formation of a rough membrane from disaccharides; anti-adhesive effect against pathogens; stimulation of nonspecific immunity; inhibition of toxins; and antagonistic effect against pathogens.

S. Bearson *et al.* (2023) highlight that the effect of the yeast cell wall on the immune system is known. These properties are attributed to substances in the middle layer of the yeast cell wall, glucans. These macromolecules can stimulate certain agents of the mammalian immune system, especially the inflammatory response and the reticuloendothelial system. The mechanism of stimulation of the immune response has been described and includes the binding of a specific glucan receptor on the surface of peripheral leukocytes and extravascular macrophages. The activation of this receptor by glucan stimulates the activation of its natural defence, which leads to a cascade reaction, primarily the production of cytokines by macrophages. C. Loving *et al.* (2023) noted that glucans are recognised as "immunoamplifiers". Glucans also significantly stimulate the phagocytic function of immune system cells. At the same time, T. Hjorth *et al.* (2023) reported an increase in the number of lactobacilli and a decrease in the number of bifidobacteria in the intestines of weaned piglets after feeding yeast, while A. Zbikowska *et al.* (2022) reported a decrease in the population of coliforms.

P. Han *et al.* (2024) showed that the preservation of normal intestinal morphology occurs due to the binding and reduction of pathogen colonisation in the gastrointestinal tract, which helps to improve the integrity of the intestinal mucosa and enhances the activity of the immune system. Additional studies conducted by W. Xiao *et al.* (2022) show that oral administration of *Saccharomices cerevisiae* to rats significantly enhances the synthesis of Ig A and the secretory component of immunoglobulins. Other studies by M. Marco *et al.* (2021) demonstrated the stimulating effect of Saccharomyces cerevisiae yeast on the content of immunoglobulins G and A in sow colostrum, which has a positive effect on the health and adaptive capacity of piglets during the early postnatal period. R. Domingos *et al.* (2021) determined that the addition of *Saccharomices cerevisiae* yeast as a probiotic to sow feed in the third trimester of pregnancy and during lactation reduced live weight loss at weaning, improved lactation, which had a positive effect on piglet weaning weight.

Therefore, it is of considerable interest to study the combined effect of the probiotic properties of yeast and a complex of enzymes on the body of sows and offspring. The study aimed to determine the effect of a feed additive containing *Saccharomyces cerevisiae* yeast and a complex of enzymes (protease, cellulase, xylanase, γ-amylase, β-glucanase, phytase) on the state of T- and B-cell specific defence and the activity of natural mechanisms of nonspecific resistance in sows and piglets born from them at critical periods of ontogeny.

MATERIALS AND METHODS

The study was conducted at the pig farm "Barkom" LLC, Sambir district, Lviv region. According to the principle of analogues, two groups of gestating sows of the Large White breed (2-3 farrowing) were formed, 8 animals in each group – control and experimental. The animals were kept in the same box, under the same feeding conditions and microclimate, which was maintained by an automated ventilation system. During the preparatory period (lasting one month), sows were kept in individual pens with individual drinkers and feeders. At the end of the preparatory period on the 85th day of farrowing, sows of the control group continued to receive standard feed (SF) balanced in terms of basic nutrients. The sows of the experimental group were additionally fed the feed additive "EnzActive Mix" in an amount of 0.3 kg/t of finished feed along with the SF. Five days before the scheduled farrowing, sows of both groups were transferred to the maternity ward, where they were kept in individual pens designed for parturition. Since then, the animals have been fed lactating sow feed. Sows of the experimental group received the feed additive "EnzActive Mix" in the amount of 0.3 kg/t of finished feed in addition to SF. After farrowing, the sows of the experimental and control groups were kept according to the specified feeding scheme. The experiment was started on the 85th day of sow farrowing and completed on the 28th day of lactation. The duration of the experiment was 58 days. During the experiment, the clinical condition of the animals was monitored, and the weight of the sows was determined by weighing them before farrowing and on the day of weaning. Feed consumption by sows during lactation was monitored.

Piglets born from sows of the control and experimental groups, starting from 5 days of age, received pre-starter and later starter feed in the corresponding periods of growth. At the same time, piglets of the experimental group were additionally fed the investigated feed additive in an amount of 0.5 kg/t of finished feed. The investigated feed additive EnzActive Mix is a unique combination of live yeast of the genus *Saccharomyces cerevisiae* and a complex of enzymes located under the layer of inactivated yeast cells. In particular, the product contains live yeast of the genus *Saccharomyces cerevisiae* with an activity of at least 1.5×10^10 CFU/g, as well as a complex of six enzymes: protease, cellulase, xylanase, α-amylase, β-glucanase, phytase (Patent No. 157586, TU U 10.9-00383320-029:2024).

The material for immunological studies was blood taken from sows from the jugular vein on the 85th and 112th day of pregnancy and the 21st day of lactation. In piglets born from sows of the experimental and control groups, blood was taken from the cranial vena cava at 5-, 14- and 28 days of age. The methods used in the study were those most suitable for the conditions described in the reference book, edition by V. Vlizlo *et al.* (2012) in the section on the study of the immunological status of animals. In heparin-stabilised blood, the total number of T-lymphocytes (T-lymphocytes) and the number of "active" T-lymphocytes (TA-RUL) were determined by the method of rosette formation with sheep erythrocytes. At the same time, their subpopulations were determined – T-helper (Th-RUL), theophylline-resistant (TR) and T-suppressor (Ts), theophylline-sensitive T-lymphocytes. The number of B-lymphocytes (EAC-RUL) is determined in the complementary rosette reaction with sheep erythrocytes. The presence of various markers and receptors on the surface of lymphocytes allows them to be differentiated from each other. The activity of rosette formation was determined by the density of receptors: 3-5 – receptors with low density; 6-10 – receptors with medium density; rosettes in the form of "morula" – receptors with high density. The number of T-cells with predominantly suppressor activity (TSA) was calculated by subtracting the number of theophylline-resistant T-cells (TR) from the total number of T-lymphocytes. The immunoregulatory index (IRI) was calculated as the ratio of theophylline-resistant T cells to theophylline-sensitive T cells. Indicators of phagocytosis of blood neutrophil granulocytes: phagocytic activity (PA), phagocytic number (PN) and phagocytic index (FI) were determined by the method of V.S. Guestev 1950 (Luzyk *et al.*, 2018).

In blood serum, the lysozyme activity (LASK) to the daily culture of *Micrococcus lysodeikticus* (strain VKM-109) was determined using the photonephelometric method, bactericidal activity (BASK) by a modified method, and the content of circulating immune complexes (CIC). All experimental animals were subjected to the least amount of stress and pain during the experiment. The blood sampling sites were treated with 70% alcohol to prevent infections from entering the injection sites. During the research, the requirements of ethical treatment of animals used in experimental studies were fully complied with (European convention for the protection of…, 1986; ISO/IEC 17025:2005, 2006). Statistical processing of the research results was performed using the standard Statistica package in Microsoft Excel 2013, assessing the reliability of the indicators ($p < 0,05$; $p < 0,01$; $p < 0,001$) by the Student's criterion.

RESULTS AND DISCUSSION

The results of the studies showed that feeding sows during the last month of pregnancy and lactation with a feed additive containing *Saccharomyces cerevisiae* yeast and a complex of enzymes had a significant effect on the activity of natural defence mechanisms (Table 1). In all periods of research after the use of the feed additive, the bactericidal and lysozyme activity of blood serum in sows of the experimental group was higher than in the control group. At the same time, the differences were more pronounced in the study of such an integral indicator of the humoral link of nonspecific resistance as the bactericidal activity of blood serum. Thus, the SBA in the blood of sows of the experimental group on the 112th day of farrowing and the 21st day of lactation was 3 ($p < 0.05$) and 4.8% ($p < 0.01$) higher, respectively, than in the animals of the control group. In the study of serum lysase activity, a significant increase (by 12%) in sows of the experimental group compared to the control group on the 112th day of pregnancy ($p < 0.001$) is noteworthy.

***Table 1**. Humoral factors of natural resistance in the blood of sows under the use of a feed additive (M ± m, n = 5)*

Indicators	Groups	Study period		
		before application	after application	
		85-day farrowing period	112-day farrowing period	21 days of lactation
LASK,	C	34.20 ± 1.50	30.00 ± 1.05	34.40 ± 1.63
	D	37.60 ± 0.93	42.00 ± 0.95***	37.80 ± 1.39
BASK,	C	31.10 ± 0.98	30.77 ± 0.41	31.78 ± 0.64
	D	34.04 ± 0.80	33.33 ± 0.83*	36.35 ± 1.17**
CIC, mmol/l	C	74.67 ± 2.60	80.20 ± 2.08	85.40 ± 2.29
	D	78.00 ± 1.70	77.20 ± 1.39	75.40 ± 3.57*

***Note:** differences are statistically significant to animals of the control group - *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

These data indicate a stimulating effect of the studied factors contained in the feed additive on the activity of humoral factors of nonspecific resistance. Instead, the study of other natural defence factors, particularly the content of circulating immune complexes, revealed a significant decrease (by 11.7%) in the blood of sows of the experimental group on the 21st day of lactation ($p < 0.05$). The formation of immune complexes in the body is the result of the specific interaction of antigens with antibodies. A gradual increase of CIC in the blood of sows of the control group was recorded with an increase in the gestation period and during lactation. The results of these studies indicate the inhibitory effect of feed additive factors on the level of circulating immune complexes in the blood of sows of the experimental group. This is a positive factor for the body since an increase in the content of CICs and their long-term circulation in the body of animals can lead to systemic or organ pathology, as indicated by V. Zhurenko *et al.* (2021), C. Cacheiro-Llaguno *et al.* (2021).

According to the authors of A. Krogh (2019), the immune system is a complex of organ and tissue defences that, together with other body systems, maintain a specific homeostasis of the internal environment. This function of the immune system is carried out through the cooperative interaction of natural and specific defence mechanisms. The last trimester of pregnancy in sows, as noted by M. Ballester *et al.* (2023), is one of the critical periods that significantly affects the state of the immune system of their body, as well as the offspring. Violation of the formation of the immune response in the body of gestating sows leads to the production of offspring with signs of congenital immunodeficiency. Given this, it is necessary to use appropriate immunotropic agents, in particular in the form of feed additives, to restore metabolic homeostasis and the immune function of the animal body.

Similar results were observed in the determination of humoral factors of natural defence in piglets born from sows of the control and experimental groups (Table 2). However, the differences were less pronounced, especially concerning such indicators as serum lysozyme activity and CIC content. There were only trends towards an increase in lysosomal activity and a decrease in the content of CIC in the blood serum of piglets of the experimental group compared to the control group. At the same time, the use of the feed additive had a significant effect on the bactericidal activity of blood serum in the experimental group. Thus, as can be seen from the data presented in Table 2, in all periods of the study, the BASK in piglets of the experimental group was higher compared to the control group. At the same time, the differences were significant at 14 and 28 days of age ($p < 0.05$). Thus, feeding piglets of the experimental group with a pre-starter feed of *Saccharomyces cerevisiae* yeast and a complex of enzymes has an activating effect on such an integral indicator of natural resistance as the bactericidal activity of blood serum. This is positive given that the studies by E. Heuß *et al.* (2019) confirm that the synthesis of humoral immunity factors in piglets increases in the early stages of their development, especially by the age of two months. A gradual increase in LAC in piglets with age was also recorded compared to the values of this indicator in newborns. In monogastric animals, the mechanism of action of *Saccharomyces cerevisiae* yeast is explained by the fact that its addition to the diet stimulates the formation of disaccharides on cell membranes, with a non-adhesive effect against pathogens, activation of nonspecific defence, weakening of toxins, and an anthoganistic effect against pathogens. The competition between yeast and pathogens for binding to intestinal cells may explain the positive effects of yeast, from adhesion to cytopathogenic effects on cells. M. Tintoré *et al.* (2023) confirmed that the constant presence of the active serotype of *Saccharomices cerevisiae* in the piglet digestive tract has an immunostimulatory effect and can protect piglets against immunological challenges.

***Table 2**. Humoral factors of natural resistance of young piglets with the use of feed additive (M ± m, n = 5)*

Indicators	Groups	Study period		
		before application	after application	
		5 days after birth	14 days after birth	28 days after birth
LASK,	C	38.67 ± 0.88	34.20 ± 1.49	41.0 ± 0.71
	D	41.00 ± 2.08	37.6 ± 0.93	42.60 ± 0.51
BASK,	C	28.71 ± 0.78	28.81 ± 0.48	29.5 ± 0.28
	D	32.08 ± 1.53	32.60 ± 1.36*	33.66 ± 1.56*
CIC, mmol/l	C	72.67 ± 2.03	66.20 ± 4.45	68.60 ± 2.99
	D	70.67 ± 2.03	62.8 ± 2.56	67.0 ± 1.58

***Note:** differences are statistically significant concerning animals of the control group - *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

To objectively assess the level of natural and adaptive immunity in animals, it is known that first- and second-level tests should be used. Given this, the authors of the study determined the cellular factors of specific immunity. The functional basis of the animal immune system is immunocompetent blood cells – lymphocytes. They are carriers of immunological memory and precursors of antibody-forming cells. Researchers such as H. Sun *et al.* (2022) believe that immunity decreases during pregnancy because the natural mechanism of its suppression, namely cellular immunity, is activated during this period. The gestational period is considered a biological model of the process of periodic or permanent immunisation of a pregnant female with autoantigens. The normal course of pregnancy is primarily associated with the immune recognition of semihalogenated foetal and placental antigens by the mother's immunocompetent cells. The mother's T-lymphocytes recognise foetal antigens. This antigen-specific immune response to paternal antigens leads to the proliferation and accumulation of individual T-cell clones. In many studies of T lymphocyte subpopulations conducted by M. Ballester *et al.* (2023), a significant decrease in the number of T helper and T suppressor cells was found with an increased value of the T suppressor/T helper ratio. T-cell function, as assessed by their colony-forming activity and proliferative response to antigen stimulation, decreases. The B-cell response and immunoglobulin production were also found to be suppressed, with a decrease in the total number of B-lymphocytes.

H. Struemper *et al.* (2022) found an increase in B-lymphocyte function accompanied by an increase in serum immunoglobulin levels. According to K. Rio-Aige *et al.* (2021), immunoglobulins in pregnant females transfer from the blood to the mammary gland shortly before delivery and can reach a concentration there ten times higher than their blood level. In these conditions, as a result of a decrease in the effector and helper functions of T cells, the risk of developing infections and malignant tumours increases, and a violation of the suppressor function of T lymphocytes leads to the appearance of "forbidden" lymphocytes clones, loss of autotolerance and the development of autoimmune diseases. The mechanisms for maintaining the body's immune homeostasis formed in the course of evolution are often unable to perform their functions, which leads to the emergence of immune pathology, mostly immunodeficiency. Therefore, it is advisable to use immunostimulating drugs during pregnancy to increase the overall resistance of the animal body.

Determination of the quantitative composition of T- and B-lymphocytes in the blood of sows and piglets born from them was carried out to assess the level of specific immunity. The data in Table 3 show that the number of T-cells and their populations in the blood of sows of the control group did not change significantly during all physiological periods of the study. Instead, some statistical changes were recorded in the blood of sows of the experimental group, which were fed a feed additive in addition to standard feed. In particular, the total number of TE-RUL in the blood of sows of the experimental group on the 112th day of pregnancy and on the 21st day of lactation was 3.6 ($p < 0.01$) and 4.2% ($p < 0.001$) higher, respectively, than in the control group. At the same time, the increase in the total number of T-lymphocytes in the blood of sows of the experimental group during the indicated periods of research occurred against the background of a probable increase in low-avid TE-RUL and a decrease in functionally inactive cells. Differences in other populations of TE-RULs in the blood of sows of the experimental group compared to the control group were not significant.

***Table 3**. The number of T-cell lymphocytes in the blood of sows with the use of feed additive, (M ± m, n = 5)*

Indicators	Groups of animals	Study period		
		before application	after application	
		85-day farrowing period	112-day farrowing period	21st day lactation
TE-RUL, 0	C	59.00 ± 1.04	58.60 ± 0.87	58.4 ± 0.50
	D	57.40 ± 0.50	55.00 ± 0.44**	54.2 ± 0.37***
3-5	C	35.80 ± 1.15	36.40 ± 0.81	37.4 ± 0.67
	D	36.80 ± 1.01	39.60 ± 0.92*	41.0 ± 0.70**
6-10	C	3.60 ± 0.24	3.60 ± 0.24	3.00 ± 0.31
	D	3.80 ± 0.37	4.00 ± 0.32	3.80 ± 0.37
M	C	1.60 ± 0.40	1.40 ± 0.24	1.20 ± 0.2
	D	2.00 ± 0.44	1.40 ± 0.24	1.00 ± 0.44
%	C	41.00 ± 1.04	41.4 ± 0.87	41.60 ± 0.50
	D	44.60 ± 0.50	45.0 ± 0.44**	45.80 ± 0.37***

***Note:** TE-RUL – total T-lymphocytes, *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

Similar changes, but expressed to a lesser extent, were noted in the study of T-active lymphocytes in the blood of sows of the experimental group under the influence of the feed additive (Table 4). Thus, on the 112th day of pregnancy and the 21st day of lactation, a significantly higher total number of T-active lymphocytes was found in the blood of sows of the experimental group compared to the control group. The increase in the number of TA-RUL in the blood of sows of the experimental group was accompanied by a simultaneous increase in the proportion of T-active lymphocytes with low avidity and a decrease in inactive TA-RUL ($p < 0.05$; $p < 0.01$). These data indicate a stimulating effect of the studied feed additive on the number of total and active T-lymphocytes in the blood. At the same time, an increase in the number of low-avidity TE-RUL was recorded in the blood of sows of the experimental group, indicating the activating effect of the factors contained in the additive on the functional state of the receptor apparatus of immunocompetent cells.

***Table 4**. The number of T-active lymphocytes in the blood of sows with the use of feed additive, (M ± m, n = 5)*

Indicators	Groups of animals	Study period		
		before application	after application	
		85-day farrowing period	112-day farrowing period	21st daylactation
TA-RUL, 0	C	76.60 ± 0.92	76.20 ± 0.86	75.20 ± 0.37
	D	74.20 ± 0.58	73.6 ± 0.40*	72.80 ± 0.37**
3-5	C	17.20 ± 1.11	19.60 ± 0.74	19.60 ± 0.67
	D	19.20 ± 0.73	21.20 ± 0.48	21.60 ± 0.64

Table 4. Continued

Indicators	Groups of animals	Study period		
		before application	after application	
		85-day farrowing period	112-day farrowing period	21st daylactation
6-10	C	5.20 ± 0.58	3.20 ± 0.37	4.80 ± 0.66
	D	5.80 ± 0.58	4.20 ± 0.37	4.40 ± 0.50
M	C	1.00 ± 0.31	1.00 ± 0.31	0.40 ± 0.24
	D	0.8 ± 0.37	1.00 ± 0.31	1.20 ± 0.20
%	C	23.40 ± 0.92	23.80 ± 0.86	24.80 ± 0.37
	D	25.80 ± 0.58	26.40 ± 0.40*	27.20 ± 0.37**

Note: *TA-lymphocytes – total T-lymphocytes, *P < 0.05, **P < 0.01, ***P < 0.001*
Source: *authors' development*

In the study of individual regulatory subpopulations of T-lymphocytes in the blood of sows under the influence of the feed additive, similar changes were also obtained (Table 5). A significant increase in the total number of theophylline-resistant T-lymphocytes in the blood of sows of the experimental group was recorded, especially on the 21st day of lactation. At the same time, on the 112th day of farrowing in the blood of sows of this group, a significantly higher number of Th-RUL with medium receptor density was found, and on the 21st day of lactation – low avidity, against the background of a decrease in the proportion of inactive forms. Under the influence of the studied feed additive, a tendency to increase the number of theophylline-sensitive subpopulations of T-lymphocytes was revealed. These changes in the number of T-lymphocytes in the blood of sows of the experimental group led to an increase in the immunoregulatory index, which characterises the ratio of T-helper to T-suppressor, but the differences were not significant. At the same time, the results of these studies indicate the activating effect of feed additive factors on the receptor apparatus of T lymphocytes by redistributing avidity and expanding the receptor field of immunocompetent cells.

Table 5*. The amount of T-helper and T-suppressor in the blood of sows with the use of feed additive, (M ± m, n = 5)*

Indicators	Groups of animals	Study period		
		before application	after application	
		85-day farrowing period	112-day farrowing period	21st day lactation
Th-RUL, 0	C	76.40 ± 0.58	76.4 ± 0.50	75.00 ± 0.54
	D	76.20 ± 0.86	73.6 ± 0.50**	72.00 ± 0.54**
3-5	C	20.00 ± 0.54	19.8 ± 1.01	21.2 ± 0.48
	D	22.60 ± 1.20	21.0 ± 0.70	24.80 ± 0.86**
6-10	C	2.60 ± 0.24	3.0 ± 0.31	2.0 ± 0.31
	D	3.00 ± 0.31	4.4 ± 0.24**	2.2 ± 0.37
M	C	0.60 ± 0.24	0.80 ± 0.37	1.00 ± 0.32
	D	0.60 ± 0.40	1.00 ± 0.32	1.00 ± 0.32
%	C	23.20 ± 0.58	23.60 ± 0.50	24.2 ± 0.37
	D	24.20 ± 0.86	26.40 ± 0.50**	28.0 ± 0.54***
T-suppressors	C	17.60 ± 1.03	17.60 ± 1.40	16.80 ± 0.58
	D	20.40 ± 1.73	18.00 ± 0.77	18.60 ± 1.24
IRI	C	1.34 ± 0.098	1.38 ± 0.144	1.44 ± 0.06
	D	1.40 ± 0.600	1.47 ± 0.058	1.50 ± 0.01

Note: *Th-RUL, IRI – immunoregulatory index; *P < 0.05, **P < 0.01, ***P < 0.001*
Source: *compiled by the authors*

The study of the number of B-lymphocytes (EAC-RUL) and the determination of their functional activity characterises the level of the humoral link of immunity. The analysis of the data in Table 6 shows that the use of a feed additive in sows during pregnancy and lactation had a similar effect on the number of B lymphocytes and their functional state as in the study of T lymphocytes and their regulatory populations. However, these changes were more pronounced. In particular, the number of B-lymphocytes in the blood of sows on the 112th day of farrowing and the 21st day of lactation was 4.6 ($p < 0.001$) and 5.2% ($p < 0.001$) higher, respectively, than in the control group. Regarding the degree of differentiation of this cell population, an increase in the number of low avid B-lymphocytes ($p < 0.01$-0.001) and a decrease in undifferentiated cells ($p < 0.001$) were observed in the blood of sows of the experimental group compared to their number in animals of the control group.

***Table 6**. The number of B-lymphocytes in the blood of sows with the use of feed additive, (M ± m, n = 5)*

Indicators	**Groups of animals**	**Study period**		
		before application	**after application**	
		85-day farrowing period	**112-day farrowing period**	**21st day lactation**
EAC-RUL, 0	C	59.60 ± 0.50	59.20 ± 0.37	59.00 ± 0.31
	D	59.00 ± 0.44	54.60 ± 0.24***	53.80 ± 0.37***
3-5	C	28.60 ± 0.67	31.70 ± 0.96	31.60 ± 0.50
	D	30.00 ± 0.84	36.60 ± 0.67**	37.60 ± 0.92***
6-10	C	7.60 ± 0.40	6.20 ± 0.37	7.40 ± 0.50
	D	7.40 ± 0.50	6.60 ± 0.50	6.60 ± 0.50
M	C	4.20 ± 0.37	2.80 ± 0.58	2.00 ± 0.31
	D	3.60 ± 2.24	2.20 ± 0.37	2.000 ± 0.31
%	C	40.40 ± 0.51	40.80 ± 0.37	41.0 ± 0.31
	D	41.00 ± 0.44	45.40 ± 0.24***	46.20±0.37***

***Note:** EAC-RUL – B lymphocytes, *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

Thus, the positive effect of the studied factors contained in the feed additive on the state of B-cell immunity in sows in different physiological periods was established. In particular, their stimulatory effect on the number of B lymphocytes and their functional activity was established. According to P. Han *et al.* (2024), an increase in the number of B-lymphocytes in the blood of sows during periods of low immune potential of their body is a positive phenomenon, as these immunocompetent cells largely provide humoral immunity by synthesising specific antibodies (immunoglobulins) and also affect the activity of some populations of T-lymphocytes in cellular immunity reactions.

When determining the state of T- and B-cell links of specific defence in the blood of piglets obtained from sows of the control and experimental groups, a similar pattern of changes in the quantitative and functional state of immunocompetent cells was revealed, especially at 14 and 28 days of age (Table 7). The results of the studies showed that the total number of T-lymphocytes and their regulatory populations in the blood of piglets of the experimental group at 5 days of age did not differ significantly from the control group, indicating that there was no significant effect of feed additive factors on the formation of the cellular link of transcolostral immunity in sows and the state of the T- and B-cell immunity of piglets. Instead, in the blood of piglets of the experimental group at 14 and 28 days of age, the number of TE-RULL was significantly higher than in the control group. At the same time, a significantly higher number of low-avidity and a lower number of inactive T-ROOL were recorded during these periods of research.

***Table 7**. The amount of TE-RUL in the blood of piglets with the use of feed additive, (M ± m, n = 5)*

Indicators	**Groups of animals**	**Study period**		
		before application	**after application**	
		5 days after birth	**14 days after birth**	**28 days after birth**
TE-RUL, 0	C	69.33 ± 0.33	69.20 ± 0.37	69.0 ± 0.44
	D	68.33 ± 0.89	73.60 ± 2.11	66.8 ± 0.73*
3-5	C	26.0 ± 0.57	26.60 ± 0.25	27.0 ± 0.54
	D	27.33 ± 0.33	28.60 ± 0.67*	28.8 ± 0.37*
6-10	C	3.33 ± 0.33	2.80 ± 0.20	3.00 ± 0.31
	D	2.66 ± 0.33	2.60 ± 0.40	2.8 ± 0.37
M	C	1.33 ± 0.33	1.40 ± 0.24	1.00 ± 0.31
	D	1.66 ± 0.33	1.20 ± 0.20	1.20 ± 0.20
%	C	30.66 ± 0.33	30.80 ± 0.37	31.0 ± 0.44
	D	31.66 ± 0.88	32.40 ± 0.50*	32.8 ± 0.37*

***Note:** TE-RUL – total T-lymphocytes, *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

A similar pattern of changes, but expressed to a lesser extent, was recorded when determining the number of T-active lymphocytes in the blood of piglets (Table 8). It should be noted that at 5 and 14 days of age, only trends in these changes were detected in the blood of piglets of the experimental group. At the same time, at 28 days of age, a significant increase in the number of T-active lymphocytes was recorded in the blood of piglets of this group against the background of a decrease in inactive TA-RUL.

***Table 8**. The number of TA-RUL in the blood of piglets with the use of feed additive, (M ± m, n = 5)*

Indicators	Groups of animals	Study period		
		before application	after application	
		5 days after birth	14 days after birth	28 days after birth
TA-RUL, 0	C	79.33 ± 0.66	78.6 ± 0.60	78.0 ± 0.70
	D	79.0 ± 0.15	77.4 ± 0.50	75.6 ± 0.74*
3-5	C	15.33 ± 1.33	17.8 ± 0.86	17.8 ± 0.86
	D	16.2 ± 0.73	19.2 ± 0.66	19.4 ± 0.67
6-10	C	4.33 ± 0.57	3.0 ± 0.54	3.6 ± 0.24
	D	4.0 ± 0.57	2.6 ± 0.24	4.0 ± 0.25
M	C	1.0 ± 0.0	0.60 ± 0.24	0.60 ± 0.24
	D	0.66 ± 0.33	0.80 ± 0.20	1.00 ± 0.31
%	C	20.66 ± 0.66	21.4 ± 0.50	22.0 ± 0.70
	D	21.0 ± 1.15	22.6 ± 0.50	24.4 ± 0.78*

***Note:** TA-RUL – total T-lymphocytes, *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

In the study of other subpopulations of T-lymphocytes (theophylline-resistant and theophylline-sensitive) in the blood of piglets under the influence of the feed additive, the differences concerning the animals of the control group were less pronounced (Table 9). At the same time, a greater number of T-helper cells and their low-avid forms in the blood of piglets of the experimental group at all periods of the study, and especially at 28 days of age, is noteworthy, but the differences concerning the control group were not significant. At all periods of the study, the IRI in the blood of piglets of the experimental group was higher than in the control group, indicating an increase in lymphocytic activity, but the differences were not significant.

***Table 9**. The number of T-helper and T-suppressor cells in piglet blood after feed additive application, (M ± m, n = 5)*

Indicators	Groups of animals	Study period		
		before application	after application	
		5 days after birth	14 days after birth	28 days after birth
T-helper, 0	C	78.66 ± 0.88	78.6 ± 1.28	78.4 ± 0.97
	D	77.0 ± 0.57	76.4 ± 0.81	76.0 ± 0.70
3-5	C	18.66 ± 0.33	19.0 ± 1.04	17.6 ± 0.87
	D	20.0 ± 1.52	20.8 ± 1.01	20.2 ± 0.96
6-10	C	2.33 ± 0.33	2.2 ± 0.24	3.4 ± 0.24
	D	2.66 ± 0.66	2.4 ± 0.24	3.2 ± 0.37
M	C	0.33 ± 0.33	0.20 ± 0.20	0.60 ± 0.24
	D	0.33 ± 0.33	0.40 ± 0.24	0.60 ± 0.24
%	C	21.33 ± 0.88	21.4 ± 1.28	21.6 ± 0.97
	D	23.0 ± 0.57	23.6 ± 0.81	24.0 ± 0.70
T-suppressors,	C	9.33 ± 0.88	9.4 ± 0.24	9.0 ± 0.94
	D	8.66 ± 1.2	9.8 ± 0.58	8.4 ± 0.67
IRI	C	2.13 ± 0.04	2.28 ± 0.17	2.50 ± 0.27
	D	1.83 ± 0.41	2.43 ± 0.12	2.93 ± 0.20

***Note:** Th-RUL, IRI – immunoregulatory index; *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

Table 10 shows the data on the determination of the number and functional activity of antigen-independent B-lymphocytes in the blood of piglets. The analysis of the study results showed that the investigated feed additive caused similar changes in the number of B-lymphocytes in the blood of early-age piglets as were found in the determination of other populations of immunocompetent cells. However, these changes were more pronounced in the blood of piglets before weaning at 28 days of age. A significantly higher number of B-lymphocytes and their high-avidity forms and a lower number of functionally inactive EAC-RUL were recorded in the blood of piglets of the experimental group compared to the control group ($p < 0.05$).

***Table 10**. The amount of EAC-RUL in the blood of piglets with the use of feed additives, (M ± m, n = 5)*

Indicators	Groups of animals	Study period		
		before application	after application	
		5 days after birth	14 days after birth	28 days after birth
EAC-RUL, 0	C	63.66 ± 0.66	63.6 ± 0.50	63.0 ± 0.31
	D	62.0 ± 0.57	62.2 ± 0.58	61.8 ± 0.37*
3-5	C	28.66 ± 0.88	27.2 ± 1.06	27.4 ± 0.40
	D	30.66 ± 0.66	29.8 ± 0.73	27.6 ± 0.67
6-10	C	5.33 ± 0.33	5.8 ± 0.73	6.0 ± 0.44
	D	5.0 ± 0.57	4.6 ± 0.24	6.4 ± 0.67
M	C	2.33 ± 0.33	3.6 ± 0.50	3.6 ± 0.24
	D	1.66 ± 0.33	3.4 ± 0.24	4.4 ± 0.24*
%	C	36.63 ± 0.66	36.4 ± 0.50	37.0 ± 0.31
	D	37.33 ± 0.33	37.8 ± 0.58	38.2 ± 0.37*

***Note:** EAC-RUL – B lymphocytes, *P < 0.05, **P < 0.01, ***P < 0.001*
***Source:** compiled by the authors*

These data indicate the activating effect of the feed additive factors on the humoral defence factors of the piglet organism in critical periods of postnatal development. The increase in the number of B-lymphocytes in the blood of piglets under the influence of the investigated feed additive at 28 days of age (before weaning) is positive, given that these immunocompetent cells, as noted above, largely provide humoral immunity by synthesising specific antibodies (immunoglobulins), and also affect the activity of some populations of T-lymphocytes in cellular immunity reactions. In general, the research results showed that the use of the EnzActive Mix feed additive in sow feed during pregnancy and lactation, as well as in piglets at an early age, has a positive effect on the immune function of the mother and offspring. This is especially important in sows in the last period of pregnancy under conditions of physiological immunosuppression and the formation of transcolostral protective factors. It is equally important for piglets, especially during critical periods of postnatal development. After all, intensive piglet-rearing technologies that involve early weaning of piglets from sows and exposure to various stress factors lead to the depletion of the body's immune and antioxidant potential and the development of immunodeficiency. It is also known that intensive livestock production technology involves not only the creation of a stable feed base but also the preservation of health and increased productivity of farm animals.

The quality of the raw materials used to make compound feed significantly impacts metabolic processes in sows during pregnancy and lactation. Poor quality grain and protein groups cause an increase in the level of free radicals and peroxide in the body of sows, which subsequently leads to a violation of metabolic homeostasis, reduced productivity and the occurrence of diseases. A significant number of enzymes is used to improve digestion and nutrient absorption of feed. According to A. Maggiolino *et al.* (2023), and T. Pruduys (2023), the use of enzyme additives significantly increases the efficiency of feed nutrient absorption and has a positive effect on piglet growth and viability. The study has demonstrated that enzyme supplements also reduce stress levels in sows during lactation. At the same time, H. Zhai *et al.* (2021) proved that the inclusion of phytase in the diet of sows improves the absorption of minerals such as calcium and phosphorus during the farrowing period. This has a positive effect on the formation of the foetal bone system and contributes to the weight gain of newborn piglets. Reducing the level of inflammation in the intestine and reducing stress levels is due to the action of the enzyme β-glucanase, which breaks down β-glucans in fibre feed. According to Zh. Li *et al.* (2021), this enzymatic action also affects the body's immune and antioxidant potential, probably through better nutrient absorption and intestinal health. An important role in this is played by microorganisms, representatives of the natural microflora of the gastrointestinal tract, which, through their active life, participate in the digestive process, counteract the development of many pathogens, and increase the body's nonspecific resistance. Probiotic preparations are developed and manufactured on their basis.

The results of the current study obtained in this experiment are consistent with the previous study by Ya. Kovalchuk *et al.* (2009), where the effect of 1 and 2% *Saccharomyces cerevisiae* yeast on the state of natural and adaptive immunity in early-age piglets and at weaning, as well as the literature on these issues (literature). The results of these studies indicate a stimulating effect of the supplementation factors on the natural mechanisms of nonspecific resistance of sows and offspring, such as serum lysozyme and bactericidal activity. This can be explained by the additive effect of biologically active substances contained in the supplement. Mannans and D-glucans have been isolated from the cell wall of the yeast *Saccharomices cerevisiae*, which exhibits immunomodulatory, radioprotective, antitumour and other properties. Recent studies demonstrated that the use of yeast as a probiotic in animal feeding has immunomodulatory properties. E. De Marco Castro *et al.* (2021) are convinced that these substances can block the attachment of pathogens to the intestinal mucosa. They may also have a versatile mechanism of action on cytokine synthesis, which makes it possible to influence immunological processes by directing their action in the Th-1 or Th-2 type. The anti-inflammatory effect of probiotics is especially relevant in diseases associated with mucosal integrity disorders, which is important for piglets in the early postnatal period of development.

S. Ogawa *et al.* (2016) demonstrated that in piglets before colostrum intake, serum immunoglobulins are absent, and their content increases dramatically due to intestinal adsorption with maternal colostrum during the first 24-36 hours of life. According to N. Martínez-Boixaderas *et al.* (2022), and D. Masiuk *et al.* (2023), in newborn piglets, cellular immunity factors are more pronounced than humoral ones in the early stages of development. This is evidenced by the appearance of a blast transformation reaction with allogeneic lymphocytes and phytohaemagglutinin, cell transplant rejection, synthesis of class M immunoglobulins, and the phenomenon of spontaneous rosette formation. However, the functional activity of cellular defence factors in this age group is also low. P. Han *et al.* (2024) noted a low level of cellular immunity in the blood of piglets up to 30 days of age, in particular, a significant number of undifferentiated and low-differentiated T-lymphocytes, a small number of T-active lymphocytes, B-lymphocytes and the concentration of certain classes of immunoglobulins, low functional activity of neutrophil granulocytes. In piglets, the differentiation of the T and B immune systems in the peripheral lymphoid organs is mainly completed by day 30 of development. At the same time, T cells bearing receptors characteristic of T helper cells make up almost 60%, and only 12-18% are T suppressors.

C. Velez *et al.* (2024) confirmed that piglets' immunity has several critical aspects, such as before colostrum consumption and from 15 to 28 days of life. During these periods, active immunity is at its lowest levels, and passive immunity is already being lost, which lasts for 4-6 weeks with sufficient colostrum consumption. This is because maternal immunoglobulins derived from colostrum begin to lose their effectiveness, and the production of their immunoglobulins is very low and increases over 7-14 days. Research results and analysis of the scientific literature show that yeast-containing probiotics are effective. This makes it possible to consider them as immunomodulatory drugs, the mechanism of action of which is mainly implemented through the mucous membrane of the digestive tract.

CONCLUSIONS

Feeding sows with the studied feed additive during periods of low immune potential of their body (in the last trimester of pregnancy and lactation), caused a stimulating effect on the activity of natural mechanisms of the humoral link of nonspecific resistance of the body. This is evidenced by the higher bactericidal and lysozyme activity of sow blood serum during pregnancy and lactation ($p < 0.05$-0.001). At the same time, on the 21st day of lactation, a lower content of circulating immune complexes was recorded in the blood of sows of this group ($p < 0.05$), indicating a decrease in the antigenic load on the animal body. In piglets born from these sows under the influence of the specified feed additive, similar changes in natural defence factors were observed but expressed to a lesser extent. A higher BASK ($p < 0.05$) and a lower CIC content ($p < 0.05$) were recorded.

The activating effect of the feed additive on the state of adaptive immunity, in particular cellular defence factors in sows and their piglets, was established. This is indicated by a higher number of T-lymphocytes (total, active and theophylline-resistant) and B-lymphocytes ($p < 0.05$-0.001) and their higher functional activity. These changes in the blood of sows and piglets were due to the redistribution of the receptor apparatus of immunocompetent cells – an increase in the number of T- and B-lymphocytes with low and medium receptor density and a decrease in functionally inactive cells ($p < 0.05$-0.001). Prospects for further research are to study the effect of Saccharomyces cerevisiae and a complex of enzymes on the antioxidant function of sows and their offspring.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the director of Barkom LLC V.M. Kuibida and veterinarian D.O. Petrusyan for the opportunity and assistance in conducting the study based on this farm. Many thanks to Enzym LLC for providing the feed additive EnzActive Mix for the study. I would also like to thank the staff of the Immunology Laboratory of the Institute of Animal Biology of the National Academy of Sciences of Ukraine for conducting immunological blood tests.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this paper.

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Стан Т- і В-клітинної ланок специфічного імунітету й активність гуморальних факторів природного захисту свиноматок та їх поросят за впливу комплексу дріжджів *Saccharomyces cerevisiae* та ферментів

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Анотація. Незрілість імунної системи новонароджених поросят призводить до значних їх втрат у постнатальний період та повністю залежить від материнського організму, особливо в останній період поросності, що зумовлено посиленням метаболічних процесів і фізіологічною імуносупресією. Мета роботи – визначити активність природних й адаптивних факторів захисту у свиноматок та народжених від них поросят за впливу кормової добавки, що містить дріжджі *Saccharomyces cerevisiae* та ферменти. Результати досліджень показали, що згодовування свиноматкам у період поросності і лактації, а також поросятам у період до відлучення кормової добавки позитивно впливало на імунну функцію організму матері та приплоду. Зокрема за дії вказаних чинників добавки констатовано активуючий вплив на природні механізми гуморальної ланки неспецифічної резистентності організму, особливо у свиноматок. Про що свідчать вища бактерицидна і лізоцимна активність сироватки крові свиноматок у період поросності і лактації ($p < 0{,}05$-$0{,}001$) та менший вміст циркулюючих імунних комплексів ($p < 0{,}05$), що вказує на зменшення антигенного навантаження на організм матері, особливо у період імуносупресії. Подібні зміни, тільки виражені меншою мірою, зафіксовано у поросят, виявлено вищу БАСК ($p < 0{,}05$) та менший вміст ЦІК ($p < 0{,}05$). Згодовування свиноматкам та поросятам дріжджів та ферментів у періоди низького імунного потенціалу їх організму спричинило активуючий вплив на стан адаптивного імунітету, зокрема клітинних факторів специфічного захисту організму. Про що свідчать збільшення у крові кількості Т-лімфоцитів (загальних, активних і теофілін-резистентних) і В-лімфоцитів ($p < 0{,}05$-$0{,}001$) та підвищення їх функціональної активності за рахунок перерозподілу рецепторного апарату імунокомпетентних клітин – збільшення кількості Т- і В-лімфоцитів з низькою і середньою щільністю рецепторів та зменшення недиференційованих у функціональному відношення клітин ($p<0{,}05$-$0{,}001$). Таким чином констатовано активуючий вплив досліджуваних чинників кормової добавки на стан природних і адаптивних факторів захисту в організмі свиноматок та їх приплоду, що позитивно впливає на формування імунної відповіді у періоди низького імунного потенціалу їх організму

Ключові слова: поросята; дріжджі *Saccharomyces cerevisiae*; пробіотики; Т- і В-лімфоцити; імунологічні дослідження