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# The status of T- and B-cell factors of specific immunity in piglets at an early age under the essential oils blend influence

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A key focus of modern pig farming is the search for natural alternatives to antibiotic growth promoters that would be capable of maintaining animal productivity and supporting the development of their immune systems. The objective of the study was to assess the effects of the phytogenic feed additive AppetiX, containing carvacrol, cinnamaldehyde, capsaicin and allicin, on the parameters of the T- and B-cell components of specific immunity in early-stage piglets. The suppelemt was given to sows during late gestation and lactation periods and to piglets as part of a pre-starter compound feed. The study was conducted on sows and their piglets, which were divided into a control group and two experimental groups. Sows in the experimental groups were fed AppetiX at a rate of 0.1 kg/t of compound feed, whilst the piglets were fed 0.1 and 0.2 kg/t of pre-starter compound feed. The intake of the supplement led to an increase in the number of total and active T-lymphocytes, a rise in the proportion of theophylline-resistant T-cells and the immunoregulatory index, whilst reducing the number of T-suppressor cells. The most pronounced changes were observed in piglets of the E2 group, which received 0.2 kg/t of the supplement. In these animals, on the 33rd day of life, the number of active T-lymphocytes was 7.0% higher, the number of theophylline-resistant T-cells was 7.1% higher, and the immunoregulatory index was 2.1 times higher than in the control group. At the same time, an increase in the number of B-lymphocytes and their functionally active forms was observed, accompanied by a decrease in the proportion of inactive cells. The results obtained indicate a positive dose-dependent effect of the phytogenic composition on the development of the cellular and humoral components of adaptive immunity in piglets at an early age. Further study of the effect of individual components of essential oils on the mechanisms regulating the immune response and the resistance of piglets in the post-weaning period holds promise.

*Keywords*: piglets; sows; phytogenic additives; carvacrol; allicin; T-lymphocytes; B-lymphocytes; immunoregulatory index; CD4/CD8 ratio.

## Introduction

Maintaining the piglets' health, particularly during critical periods of their growth and development, is one of the key challenges in modern intensive pig farming. The early postnatal period in piglets is the stage during which their immune system matures and they adapt to external environmental factors. During this period, animals are particularly susceptible to infectious diseases and stress factors, which in most cases lead to a reduction in their vitality, slower growth, and increased mortality (Pomorska-Mól et al., 2013; Gresse et al., 2017; Maes et al., 2020). Data analysis from recent studies has shown that the immune system of piglets develops under the dual influence: on the one hand, the immunoglobulins intake, cellular and non-globulin defence factors, as well as other biologically active substances via the sows' milk (Krolikowski et al., 2021), and on the other hand, through the development of their own immune factors, including the T- and B-lymphocytes activation, which are responsible for the cellular and humoral components of immunity (Poonsuk & Zimmerman, 2018; Szabó et al., 2025).

In conditions of the ban on the use of antibiotics as growth promoters in animal husbandry (Regulation (EC) No 1831/2003), researchers are focusing considerable attention on natural alternatives, among which phytogenic additives and essential oils hold a special place. These contain a wide range of biologically active compounds (carvacrol, cinnamaldehyde, allicin, and capsaicin) which exert antimicrobial, anti-inflammatory, and immunomodulatory effects (Rivera-Gomis et al., 2020; Deng et al., 2023; Prudyus et al., 2024; Nasser et al., 2025). Recent studies have shown that individual components of phytogenic compositions can directly influence the functional state of adaptive immune cells. In particular, carvacrol and other phenolic compounds in oregano essential oil are capable of regulating the expression of pro-inflammatory cytokines, stimulating T-lymphocyte proliferation and supporting the functional activity of antigen-presenting cells (Rivera-Gomis et al., 2020; Duarte & Kim, 2022). Allicin, the main bioactive compound in garlic, enhances the proliferation of T-helper cells, the activity of macrophages, and the production of interleukins IL-2 and IFN-γ, thereby promoting the development of a cellular immune response. Allicin is capable of stimulating the proliferation of CD4+ T-lymphocytes and increasing the production of IL-2 and IFN-γ, whilst carvacrol enhances the activation of CD3+ and CD4+ cells and regulates the cytokine profile of the immune response (Imran et al., 2022). Furthermore, both compounds can activate the humoral component of immunity by stimulating B-lymphocytes and the synthesis of IgG and IgA immunoglobulins (Savairam et al., 2023; Zhang et al., 2023; Wang et al., 2024). Cinnamaldehyde and capsaicin are characterised by pronounced antioxidant properties (Guo et al., 2024) and the ability to modulate the NF-κB and MAPK signalling pathways, thereby reducing excessive inflammatory responses and creating favourable conditions for the differentiation and function of T and B lymphocytes. In addition to their direct effects on immunocompetent cells, these compounds may indirectly enhance specific immunity by normalising the gut microbiota and improving the intestinal barrier function (Periferakis et al., 2023). It is known that the state of the gut microbiome is closely linked to the maturation of lymphoid tissue associated with the intestinal mucosa and the formation of T- and B-cell populations in young pigs (Gresse et al., 2017; Han et al., 2024). Therefore, the use of multi-component phytogenic formulations can be considered a promising approach to stimulating the development of adaptive immunity in young piglets. In view of this, the use of phytogenic formulations in animal feed may not only help to improve digestion and productivity (Prudyus, 2024), as well as nutrient utilisation, but also contribute to the development of a robust immune response (Windisch et al., 2008; Han et al., 2024).

Particular attention should be paid to the strategy of using phytogenic preparations not only directly in piglets' diets, but also in the sows' feed. There is evidence that the composition of sow feed significantly influences the immune status of the litter by altering the milk composition, in particular the content of immunoglobulins, cytokines, and other regulatory molecules (Mirpuri et al., 2021; Santos, 2023; Prudyus et al., 2024).

In this context, the use of phytogenic formulations in the sow diets during the final stage of gestation and during lactation may prove to be an effective means of enhancing the transcolostral immune factors in piglets at an early age.

However, the data in the scientific literature are fragmentary and require comprehensive study and synthesis. Therefore, the objective of the research was to investigate the effect of adding a microencapsulated phytogenic formulation to the diet of sows and their litters on the status of the T- and B-cell components of the piglets' immune system.

**Materials and methods**

All animals used in the experiment were subjected to the minimum possible stress and pain. The housing and feeding of the sows and piglets complied with current zootechnical requirements. All procedures adhered to the 'General Ethical Principles for Animal Experimentation', adopted by the 1st National Congress on Bioethics of Ukraine in 2001. This document is consistent with international standards on the protection of animal rights and welfare in research and incorporates the principles enshrined in *The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* (Strasbourg, 1986).

The scientific and production study was carried out at one of the pig farms in Lviv Oblast on Large White sows and their piglets. Fifteen pregnant sows (2–3 lactations) were selected for the experiment and divided into three groups on a matching basis: the control group (C) and two experimental groups – Experimental Group 1 (E1) and Experimental Group 2 (E2), with five sows in each group. The feeding regime for gestating and lactating sows followed the feeding plan, whereby gestating sows were fed standard compound feed (SF) for gestating sows twice daily, whilst lactating sows were fed SF for lactating sows three times daily in the quantities specified by the ration. The animals were watered using nipple drinkers, with free, unrestricted access to water. In addition to the SC, sows in the experimental groups were given the phytogenic feed additive AppetiX at a rate of 0.1 kg/t. Piglets born by the sows were fed pre-starter compound feed (PSF) in loose form from 5 days of age. The compound feed was provided to the piglets throughout the day in plastic feeders in small portions as they ate. Every morning, any remaining feed was removed and weighed to determine actual feed intake. After cleaning, washing and drying the feeders, the feed was replenished. The piglets had free access to water, which was supplied via a bowl-type drinker. In addition to the standard feed, the animals in group E1 received 0.1 kg/t of the phytogenic supplement AppetiX, whilst those in group E2 received 0.2 kg/t. The control group of piglets received only the standard feed.

Sows and their piglets were kept under identical microclimatic conditions, in accordance with existing technical standards.

For immunological studies, blood was collected from the jugular vein of the piglets at three different ages: on the 5th day of life (before feeding with CF) and at 19 and 33 days of age. The blood collection sites on the animals were treated with 70% alcohol to prevent infection.

In heparin-stabilised blood from the piglets, the numbers of T- and B-lymphocytes and their separate subpopulations were determined using a spontaneous rosette formation reaction with sheep erythrocytes as markers, as described by us in the handbook (Vlizlo & Vishchur, 2012). The principle of the method lies in the ability of lymphocytes to form the so-called 'rosettes' with heterogeneous erythrocytes. At the centre of the 'rosette' is a lymphocyte, the membrane of which bears specific receptors, whilst erythrocytes are located at the periphery. The degree of lymphocyte activity is assessed based on the number of erythrocytes adsorbed by a single lymphocyte. The studies determined the relative numbers of total (total rosette-forming lymphocytes) and active T-lymphocytes (E-RFC – active rosette-forming lymphocytes), as well as theophylline-resistant T-lymphocytes (TFR). The number of T-cells with predominantly suppressor activity (theophylline-sensitive, TFS) was calculated by subtracting the number of theophylline-resistant T-cells from the total number of T-lymphocytes. The immunoregulatory index (CD4/CD8 ratio) was quantified as the ratio of theophylline-resistant to theophylline-sensitive T-lymphocytes. Lymphocyte differentiation is based on the presence of specific markers and receptors on their surface: T-lymphocytes are distinguished by the presence of receptors for heterogeneous erythrocytes, whilst B-lymphocytes, in addition to their own immunoglobulins, have receptors for the Fc fragment and the third component of complement (C3). It was precisely these functional characteristics that were used to determine the number of T- and B-cells in the blood of the piglets.

Buffered saline solution (pH 7.2–7.4 [7.3]) was used to wash the lymphocytes. The mononuclear cell fraction was isolated from heparinised piglet blood. The smears were air-dried, fixed with methanol, and stained for 7–10 minutes using the Romanovsky–Giemsa method. Rosettes were counted under a microscope, and rosette formation activity was assessed based on receptor density: 3–5 – low density, 6–10 – medium, 'morula-like' rosettes – high density.

The AppetiX feed supplement is a combination of natural, standardised bioactive substances extracted from aromatic herbs and spices, combined into a single microencapsulated particle to ensure stability and the gradual release of the active ingredients in the digestive tract. The supplement contains:

– oregano (*Origanum vulgare*) essential oil – the main bioactive compound, carvacrol, which exerts powerful bactericidal, fungicidal, and antioxidant properties; carvacrol effectively inhibits the growth of pathogenic bacteria (*E. coli*, *Salmonella* spp., *Clostridium* spp.) and fungi, helps to maintain the balance of the gut microbiota and strengthens the gut's protective barrier;

– cinnamon essential oil (*Cinnamomum ceylanicum*) – the main active ingredient is cinnamaldehyde, which acts as a powerful antioxidant and appetite stimulant; cinnamaldehyde activates taste and smell receptors, improves feed intake, reduces oxidative stress, and exhibits moderate antimicrobial activity, which helps to stabilise the gut microbiota;

– chilli pepper extract (*Capsicum annuum*) – the main active compound is capsaicin, which stimulates the secretion of saliva and gastric juice, activates pancreatic function, and enhances intestinal peristalsis; this improves the absorption of nutrients and reduces feed conversion; capsaicin also acts as a natural stimulant of blood circulation and has a mild anti-inflammatory effect;

– garlic (*Allium sativum*) essential oil – the main active ingredient is allicin, which has pronounced antimicrobial, antifungal, and immunomodulatory properties; allicin is capable of inhibiting the growth of pathogenic microflora in the gastrointestinal tract, reducing gas formation, stimulating the immune response, and improving metabolic processes in young animals.

Throughout the experiment, the principle of minimising stress and pain in the animals was adhered to. When collecting blood samples, the injection site was thoroughly disinfected with a 70% ethanol solution to prevent possible infection. The housing of the sows and piglets, as well as their feeding regime, complied with current zootechnical standards and veterinary requirements.

Statistical analysis of the study results was performed using one-way analysis of variance (ANOVA) with the StatPlus Pro version 8 software (AnalystSoft Inc., Walnut, California, USA). Tukey's HSD post-hoc test was used for multiple comparisons of mean values. Results are presented as mean ± SD (standard deviation). Differences between groups were considered statistically significant at $P < 0.05$.

## Results

The results of the studies showed that the use of the feed additive containing essential oils had an immunomodulatory effect on the cellular component of specific immunity in the piglets, in particular on the number of T- and B-lymphocytes and their functional activity (Table 1). At the same time, a dose-dependent effect of the feed additive on the immunocompetent blood cells under investigation was observed. This was evidenced by the higher number of T- and B-lymphocytes and their greater functional activity in animals from the second experimental group (E2), which consumed a higher dose of the additive (0.2 kg/t of compound feed). This supports the assertion that the active components of the studied supplement (plant extracts, natural bioactive compounds, polyphenols) not only stimulate digestion but also exert a systemic effect on the immune regulation of immunocompetent blood cells.

**Table 1**
The number of total T-lymphocytes (TE-RUL) and their subpopulations in the blood of the piglets following the administration of the feed additive AppetiX (%, mean ± SD, n = 5)

| Parameters | Groups of animals | Periods of experiment | | |
|---|---|---|---|---|
| | | before administration | after administration | |
| | | 5th day of life | 19th day of life | 33th day of life |
| 0 (non-rosette-forming cells) | Control | 71.00 ± 1.22[a] | 70.00 ± 0.71[a] | 67.60 ± 0.55[a] |
| | Experiment 1 | 68.60 ± 1.52[ab] | 68.20 ± 1.30[ab] | 67.40 ± 2.07[a] |
| | Experiment 2 | 67.40 ± 1.14[ac] | 66.00 ± 0.71[abc] | 64.00 ± 0.71[abc] |
| 3–5 (low-avidity T-lymphocytes) | Control | 24.00 ± 1.00[a] | 24.80 ± 0.84[a] | 26.00 ± 0.71[a] |
| | Experiment 1 | 25.80 ± 0.84[ab] | 25.60 ± 1.14[a] | 25.80 ± 1.92[a] |
| | Experiment 2 | 26.60 ± 1.14[ac] | 26.80 ± 0.84[ac] | 27.40 ± 0.89[a] |
| 6–10 (medium-avidity T- lymphocytes) | контрольна | 4.40 ± 0.55[a] | 5.20 ± 0.45[a] | 5.00 ± 0.71[a] |
| | Experiment 1 | 5.00 ± 1.00[a] | 5.60 ± 0.55[a] | 5.60 ± 0.55[a] |
| | Experiment 2 | 5.40 ± 0.55[a] | 6.00 ± 0.71[a] | 6.80 ± 0.84[abc] |
| >10 (high avidity T-lymphocytes) | Control | 0.60 ± 0.548[a] | 0.20 ± 0.45[a] | 1.40 ± 0.55[a] |
| | Experiment 1 | 0.60 ± 0.548[a] | 0.60 ± 0.55[a] | 1.20 ± 0.84[a] |
| | Experiment 2 | 0.60 ± 0.548[a] | 1.20 ± 0.84[a] | 1.80 ± 0.45[a] |
| Total number TE-RUL | Control | 29.00 ± 1.22[a] | 30.00 ± 0.71[a] | 32.40 ± 0.55[a] |
| | Experiment 1 | 31.40 ± 1.52[ab] | 31.80 ± 1.30[ab] | 32.60 ± 2.07[a] |
| | Experiment 2 | 32.60 ± 1.14[ac] | 34.00 ± 0.71[abc] | 36.00 ± 0.71[abc] |

*Note*: values within the same age group for a single indicator denoted by different letters ([a], [b], [c]) are statistically different from one another according to the Tukey test at P < 0.05; identical letters indicate that there is no statistically significant difference between the groups.

The data presented in Table 1 show that the total number of T-lymphocytes and their functional status in the blood of piglets in the experimental groups at 5 days of age do not differ significantly from those in the control group. These data indicate that feeding sows the test feed additive during the final stage of gestation had no significant effect on the functional status of T- and B-lymphocytes in the blood. At the same time, at 19 days of age, a tendency towards an increase in the total number of T-lymphocytes was observed only in the E2 piglets, whilst in the group E1 the changes were insignificant. However, at 33 days of age, the differences in the total number of T-lymphocytes in the blood of the E2 piglets compared with the control group were statistically significant (P < 0.05). It should also be noted that the total number of T-lymphocytes in the blood of piglets in the first experimental group was at the same level as in the control group, indicating a dose-dependent effect of the phytosupplement.

When assessing the functional status of T-lymphocytes in the piglets following intake of the feed supplement containing a combination of essential oils, we observed a notable upward trend in the total number of T-lymphocytes with low, medium, and high receptor densities in the blood of piglets in the second experimental group compared with the control group at 19 days of age, and particularly at 33 days of age, when the differences were statistically significant. The increase in the total number of functionally high-avidity T-lymphocytes in the blood of the E2 piglets occurred against a background of a decrease in the number of inactive T-cells. The changes observed in the blood of the E2 piglets indicate a stimulatory effect of the feed supplement's components on the total number of T-lymphocytes and their functional activity.

It is known that a highly sensitive indicator of the state of immune system's T-cell component is the number of active T-lymphocytes (E-RFC) and their subpopulations in the blood of piglets (Table 2). When examining this cell population in the blood of piglets, similar changes were observed to those seen when determining the total number of T-lymphocytes. Thus, the number of E-RFC in the E2 piglets at 19 and 33 days of age exceeded that of the control group by 6.1% (P < 0.05) and 7.0% (P < 0.01), respectively. These changes occurred alongside a simultaneous reduction in the proportion of their inactive forms and an increase in functionally highly avid active rosette-forming T-lymphocytes (E-RFC), indicating an increase in the functional maturity and metabolic activity of this population of immunocompetent cells. Under the influence of the bioactive components of essential oils, T-cell differentiation was stimulated and intercellular signalling was enhanced.

The components of the studied supplement had a stimulating effect on the receptor system and the number of theophylline-resistant T-lymphocytes in the blood of the piglets (Table 3). In particular, an increase was observed in the proportion of T-helper cells – the main regulators of the humoral response. Thus, the number of Th-lymphocytes in the E2 piglets at 19 and 33 days of age exceeded that of the control group by 4.0% (P < 0.05) and 7.1% (P < 0.01), respectively. Similar changes during these periods were also observed in the blood of the E1 piglets; however, they were less pronounced and the differences compared with the control group were not statistically significant. By contrast, the number of T-suppressor cells in the blood of piglets in the first and second experimental groups at 33 days of age decreased by 1.4 and 1.5 times, respectively (P < 0.05). Consequently, the immunoregulatory index (CD4/CD8 ratio) more than doubled in the group E2 during this period (P < 0.01), which is a sign of immune activation (Table 3). This trend indicates an enhancement of the helper function of T-cells, which ensure coordination between the cellular and humoral components of the immune response. This increase in the T-cell pool indicates the activation of lymphocyte proliferation, a strengthening of their immunoregulatory role, and an increase in the body's overall reactivity.

Table 4 presents the results of studies of the effects of the AppetiX supplement on the status of the B-lymphocyte component of specific immunity in the piglets. The data in the table show that the total number of T-lymphocytes in the blood of piglets in the experimental groups was higher than in the control group throughout all periods of the experiment, and particularly in the E2 piglets. It should be noted, however, that unlike T-lymphocytes, the number of B-lymphocytes tended to increase in the blood of the piglets as early as 5 days of age, indicating the activating effect of the combination of essential oils in the studied supplement on the humoral factors of transcolostral protection. Thus, feeding the piglets a higher dose of the additive led to an increase in the number of EAC-RFCs in the blood and an increase in their functional activity, particularly at 33 days of age. Thus, in the E2 piglets during this period, against a background of an increase in the total number of EAC-RFC (EAC rosette-forming cells) in the blood, a decrease in the number of their inactive forms was recorded, accompanied by a simultaneous increase in the proportion of B-lymphocytes with low and medium degrees of avidity ($P < 0.05$). This indicates the activation of antibody production, that is, an enhancement of the humoral immune response. As is well known, exposure to essential oils reduces the level of oxidative stress in cells, which has a stabilising effect on lymphocyte membranes, preventing their damage by free radicals. This, in turn, has a positive effect on the functional state of the receptor apparatus of immunocompetent cells, enhancing their avidity. As a result, the proliferative activity of B-lymphocytes increases, and their differentiation into plasma cells occurs more intensively. Such changes contribute to enhanced antibody synthesis and an increase in the level of specific mechanisms of humoral immunity in piglets.

**Table 2**
Number of active T-lymphocytes (E-RFC) and their subpopulations in the blood of the piglets following the administration of the feed additive AppetiX (%, mean ± SD, n = 5)

| Parameters | Groups of animals | Experiment periods | | |
|---|---|---|---|---|
| | | before administration | after administration | |
| | | 5$^{th}$ day of life | 19$^{th}$ day of life | 33$^{th}$ day of life |
| 0 (non-rosette-forming cells) | Control | 81.20 ± 0.84$^{a}$ | 79.00 ± 1.22$^{a}$ | 78.40 ± 1.14$^{a}$ |
| | Experiment 1 | 77.80 ± 4.44$^{a}$ | 78.80 ± 1.30$^{a}$ | 76.80 ± 0.84$^{ab}$ |
| | Experiment 2 | 75.60 ± 1.14$^{ac}$ | 72.80 ± 0.45$^{abc}$ | 71.40 ±1.14$^{abc}$ |
| 3–5 (low-avidity T-lymphocytes) | Control | 13.80 ± 0.84$^{a}$ | 15.20 ± 0.84$^{a}$ | 15.80 ± 0.84$^{a}$ |
| | Experiment1 | 15.20 ± 0.84$^{ab}$ | 15.60 ± 1.14$^{a}$ | 17.00 ± 0.71$^{ab}$ |
| | Experiment 2 | 17.80 ± 0.84$^{abc}$ | 19.00 ± 0.71$^{abc}$ | 20.80 ± 0.84$^{abc}$ |
| 6–10 (medium-avidity T- lymphocytes) | контрольна | 4.60 ± 0.55$^{a}$ | 4.40 ± 0.55$^{a}$ | 4.80 ± 0.84$^{a}$ |
| | Experiment 1 | 4.40 ± 0.89$^{a}$ | 4.60 ± 0.55$^{a}$ | 4.40 ± 0.55$^{ab}$ |
| | Experiment 2 | 5.40 ± 0.55$^{a}$ | 6.60 ± 0.55$^{abc}$ | 6.20 ± 0.84$^{abc}$ |
| M (>10) (high avidity T-lymphocytes) | Control | 0.40 ± 0.548$^{a}$ | 1.40 ± 0.89$^{a}$ | 0.80 ± 0.84$^{a}$ |
| | Experiment 1 | 0.80 ± 0.447$^{a}$ | 1.00 ± 0.71$^{a}$ | 1.80 ± 0.45$^{a}$ |
| | Experiment 2 | 1.20 ± 0.837$^{a}$ | 1.60 ± 0.55$^{a}$ | 1.60 ± 0.55$^{a}$ |
| Total number of active T-lymphocytes | Control | 18.80 ± 0.84$^{a}$ | 21.00 ±1.22$^{a}$ | 21.60 ± 1.14$^{a}$ |
| | Experiment 1 | 20.40 ± 0.89$^{ab}$ | 21.20 ± 1.30$^{a}$ | 23.20 ± 0.84$^{ab}$ |
| | Experiment 2 | 24.40 ± 1.14$^{abc}$ | 27.20 ± 0.45$^{abc}$ | 28.60 ± 1.14$^{abc}$ |

*Note*: see Table 1.

**Table 3**
The number of theophylline-resistant (Th) and theophylline-sensitive (Ts) T-lymphocytes and the immunoregulatory index (CD4/CD8 ratio) in the blood of the piglets following the administration of the feed additive AppetX (%, mean ± SD, n = 5)

| Parameters | Groups of animals | Experiment periods | | |
|---|---|---|---|---|
| | | before administration | after administration | |
| | | 5$^{th}$ day of life | 19$^{th}$ day of life | 33th day of life |
| 0 (non-rosette-forming cells) | Control | 79.60 ± 0.89$^{a}$ | 79.00 ± 0.71$^{a}$ | 79.00 ± 1.73$^{a}$ |
| | Experiment 1 | 77.40 ± 1.52$^{ab}$ | 76.40 ± 0.55$^{ab}$ | 75.60 ± 0.89$^{ab}$ |
| | Experiment 2 | 76.40 ± 1.14$^{ac}$ | 75.00 ± 1.41$^{ac}$ | 71.40 ± 1.14$^{abc}$ |
| 3–5 (low-avidity T-lymphocytes) | Control | 16.20 ± 0.84$^{a}$ | 16.20 ± 0.84$^{a}$ | 16.60 ± 1.14$^{a}$ |
| | Experiment 1 | 17.80 ± 0.84$^{ab}$ | 17.60 ± 0.55$^{ab}$ | 17.60 ± 1.14$^{a}$ |
| | Experiment 2 | 18.20 ± 0.84$^{ac}$ | 19.80 ± 0.84$^{abc}$ | 21.60 ± 1.14$^{abc}$ |
| 6–10 (medium-avidity T- lymphocytes) | Control | 3.80 ± 0.84$^{a}$ | 5.00 ± 0.71$^{a}$ | 4.20 ± 0.84$^{a}$ |
| | Experiment 1 | 4.40 ± 0.55$^{a}$ | 5.40 ± 0.55$^{a}$ | 5.60 ± 0.55$^{ab}$ |
| | Experiment 2 | 5.00 ± 0.71$^{ac}$ | 4.60 ± 0.55$^{a}$ | 5.60 ± 0.55$^{ac}$ |
| M (>10) (highly avid T-lymphocytes) | Control | 0.40 ± 0.55$^{a}$ | 0.20 ± 0.45$^{a}$ | 0.40 ± 0.55$^{a}$ |
| | Experiment 1 | 0.40 ± 0.55$^{a}$ | 0.60 ± 0.55$^{a}$ | 1.20 ± 0.84$^{a}$ |
| | Experiment 2 | 0.40 ± 0.55$^{a}$ | 0.60 ± 0.55$^{a}$ | 1.40 ± 0.55$^{a}$ |
| Total quantity of Th-lymphocytes | Control | 20.40 ± 0.89$^{a}$ | 21.00 ±0.71$^{a}$ | 21.00 ± 1.73$^{a}$ |
| | Experiment 1 | 22.60 ± 1.52$^{ab}$ | 23.60 ± 0.55$^{ab}$ | 24.40 ± 0.89$^{ab}$ |
| | Experiment 2 | 23.60 ± 1.14$^{ac}$ | 25.00 ± 1.41$^{abc}$ | 28.60 ± 1.14$^{abc}$ |
| Theophylline-sensitive T-lymphocytes (Ts), % | Control | 8.60 ± 1.80$^{a}$ | 9.00 ± 1.22$^{a}$ | 11.40 ± 1.52$^{a}$ |
| | Experiment 1 | 8.80 ± 2.59$^{a}$ | 8.20 ± 1.30$^{a}$ | 8.20 ± 2.28$^{ab}$ |
| | Experiment 2 | 9.00 ± 1.22$^{a}$ | 9.00 ± 2.00$^{a}$ | 7.40 ± 1.67$^{ac}$ |
| Immunoregulatory index, CD4/CD8 ratio (Th/Ts) | Control | 2.42 ± 0.43$^{a}$ | 2.36 ± 0.43$^{a}$ | 1.84 ± 0.45$^{a}$ |
| | Experiment 1 | 2.77 ± 0.91$^{a}$ | 2.90 ± 0.48$^{a}$ | 2,97 ± 0.94$^{ab}$ |
| | Experiment 2 | 2.62 ± 0.45$^{a}$ | 2.88 ± 0.72$^{a}$ | 3,86 ± 0.95$^{ac}$ |

*Note*: see Table 1.

Summarising the results of the conducted studies, it can be concluded that the feed additive containing essential oils (*Origanum vulgare*, *Cinnamomum ceylanicum*, *Capsicum annuum*, *Allium sativum*) stimulates the development of a fully-fledged immune response by simultaneously strengthening the T-cell-mediated (cellular) and B-cell-mediated (humoral) components of the piglets' immune system. Such changes are physiologically sound, with no signs of hyperstimulation, and indicate an increase in the organism's immune and adaptive potential during the period of intensive growth. These changes were more pronounced in the E2 piglets, confirming the dose-dependent effect of the preparation.

Consequently, the use of essential oils in a feed supplement in the diet of sows and piglets at an early age has a positive effect on the development of colostral defence factors, enhances the activity of the piglets' adaptive specific defence mechanisms during critical periods of neonatal growth, and ensures resistance to infections.

**Discussion**

The results obtained indicate that the herbal additive has a significant stimulatory effect on the development of the immune system in piglets during the early postnatal period. The increase in the number of total T-lymphocytes and T-active lymphocytes in the groups E1 and E2 indicates the activation of innate cellular immunity and the body's preparation for adaptation during the period of high susceptibility to infectious and stress factors. The most pronounced increase in these populations of immunocompetent cells in the group E2 reflects the dose-dependent effect of the herbal supplement in the pre-starter compound feed, confirming the need to adjust the dosage for optimal activation of the immune response in newborns, which is consistent with the findings of other researchers (Samanta et al., 2021; Duarte et al., 2022).

**Table 4**
The number of B-lymphocytes and their subpopulations in the blood of the piglets following the administration of the feed additive AppetiX (%, mean ± SD, n = 5)

| Parameters | Groups | Experimental periods | | |
|---|---|---|---|---|
| | | before administration | after administration | |
| | | 5th day of life | 19th day of life | 33th day of life |
| 0 (non-rosette-forming cells) | Control | 69.80 ± 1.10[a] | 67.00 ± 0.71[a] | 65.00 ± 1.00[a] |
| | Experiment 1 | 67.40 ± 0.89[ab] | 65.40 ± 1.41[ab] | 64.00 ± 1.58[a] |
| | Experiment 2 | 66.00 ± 1.58[ac] | 63.20 ± 0.84[abc] | 61.40 ± 1.67[abc] |
| 3–5 (low-avidity B-lymphocytes) | Control | 24.20 ± 0.84[a] | 25.80 ± 0.84[a] | 26.80 ± 1.30[a] |
| | Experiment 1 | 25.40 ± 0.55[ab] | 27.20 ± 0.84[ab] | 28.00 ± 1.00[ab] |
| | Experiment 2 | 26.20 ± 0.84[ac] | 28.40 ± 1.14[ac] | 29.40 ± 1.14[a] |
| 6–10 (medium-avidity B-lymphocytes) | Control | 4.60 ± 0.55[a] | 5.40 ± 0.55[a] | 6.60 ± 0.55[a] |
| | Experiment 1 | 4.80 ± 0.84[a] | 6.00 ± 0.71[a] | 6.40 ± 0.55[a] |
| | Experiment 2 | 5.60 ± 0.55[a] | 6.20 ± 0.84[a] | 7.20 ± 0.84[a] |
| M (>10) (highly avid B-lymphocytes) | Control | 1.40 ± 0.55[a] | 1.80 ± 0.45[a] | 1.60 ± 0.55[a] |
| | Experiment 1 | 2.40 ± 0.89[a] | 1.40 ± 0.55[a] | 1.60 ± 0.55[a] |
| | Experiment 2 | 2.40 ± 0.55[a] | 2.20 ± 0.45[a] | 2.00 ± 0.00[ab] |
| Total B-lymphocyte quantity, % | Control | 30.20 ± 1.10[a] | 33.00 ± 0.71[a] | 35.00 ± 1.00[a] |
| | Experiment 1 | 32.60 ± 0.89[ab] | 34.60 ± 1.14[ab] | 36.00 ± 1.58[a] |
| | Experiment 2 | 34.00 ± 1.58[ac] | 36.80 ± 0.84[abc] | 38.60 ± 1.67[abc] |

*Note*: see Table 1.

The increase in the number of active rosette-forming T-lymphocytes (E-RFC), T-helper cells, and the immunoregulatory index that we observed is consistent with the results of studies in which allicin and carvacrol contributed to an increase in CD3+ and CD4+ cell populations and enhanced secretion of IL-2 and IFN-γ (Imran et al., 2022). Similarly, the increase in the number of EAC-RFC (EAC rosette-forming cells) observed in our studies may be linked to the stimulation of B-cell proliferation and the synthesis of IgG and IgA, as has been described for allicin and oregano essential oil (Zhang et al., 2023; Wang et al., 2024).

An important component of the mechanism of action of the components of AppetiX is their integrated effect on the bodies of sows and their piglets. The addition of the phytosupplement to sows' compound feed at a rate of 0.1 kg/t from day 85 of gestation contributed to an improvement in the biological value of colostrum and an increase in the concentration of cellular factors and antibodies, thereby establishing passive immunity in newborn piglets (Prudyus et al., 2024). The use of the supplement in the pre-starter compound feed for piglets at an early age had a stimulating effect on the immune system, particularly enhancing the proliferation of T-lymphocytes and the activity of B-cells, which are responsible for the humoral component of immunity. This combined approach ensures synergy between passive and active immunity, accelerates the development of immune competence, and increases resistance to pathogenic influences during critical periods of development (Gresse et al., 2017).

The increase in the number of B-lymphocytes in the piglets blood of the experimental groups, particularly in the group E2, indicates an intensification of the humoral component of immunity. This reflects the phytosupplement's ability to stimulate antibody synthesis, which is critically important for protecting piglets during early postnatal development, when their own immune system is not yet fully developed. The simultaneous increase in T-active lymphocytes and the relative stability of T-suppressor cells indicate the maintenance of immune balance, the prevention of excessive reactions, and the formation of a balanced immune profile (Sinkora & Butler, 2016).

The mechanisms of action of this herbal additive are attributed to its multi-component composition, which includes essential oils, polyphenols, and other biologically active substances. These can stimulate T-lymphocyte proliferation, enhance macrophage activity, and modulate the cytokine profile, thereby promoting the rapid establishment of the 'sow–piglet' immune connection (Gresse et al., 2017; Vishchur et al., 2020). The results obtained may be linked to the specific biological properties of individual components of the AppetiX feed additive. For example, carvacrol, a component of oregano essential oil, is capable of stimulating lymphocyte proliferation, enhancing the activity of natural killer cells, and modulating the production of cytokines that regulate T-cell differentiation (Cappelli et al., 2021). Allicin activates the function of T-helper cells and macrophages, helps to enhance the cell-mediated immune response and increase antibody production (Arreola et al., 2015). Cinnamaldehyde and capsaicin, thanks to their antioxidant and anti-inflammatory properties, reduce oxidative stress (Tanaka et al., 2019), which has a positive effect on the survival and functional activity of lymphocytes.

The increase in the number of B-lymphocytes in the piglets' blood of the experimental groups may be the result of both a direct stimulatory effect of plant bioactive substances on B-cell proliferation and indirect activation via enhanced helper function of T-lymphocytes. The increase in the immunoregulatory index that we observed is consistent with the literature data regarding the ability of phytogenic additives to enhance cooperation between the cellular and humoral components of the immune system without causing excessive immune activation.

The increase in the amount of phytogenic compounds in the diet of piglets in the group E2 resulted in a greater stimulatory effect on adaptive defence mechanisms, as evidenced by the rise in the immunoregulatory index, which indicates the organism's enhanced ability to mount an immune response to an antigenic stimulus.

Our research findings are consistent with those of other researchers, who have noted the positive effect of phytosupplements on the development of the immune system in pigs and improved piglet survival (Wang et al., 2022; Madesh et al., 2025; Lykhach et al., 2026). Of particular importance is a combined approach that integrates maternal support with the early inclusion of a phytosupplement in the piglets' diet. On the one hand, this ensures the transfer of cellular and humoral components from the sow's blood to the mammary gland and, consequently, into colostrum and milk; on the other hand, it facilitates the effective development of the piglets' own immune system, which is particularly important during critical periods of their development.

## Conclusions

The inclusion of the phytogenic feed additive AppetiX in the diet of sows during late gestation and lactation, as well as in pre-starter compound feed for piglets, had a positive effect on the development of the T- and B-cell components of specific immunity.

In piglets from the experimental groups, activation of the cellular and humoral components of adaptive immunity was observed, particularly in the group E2 animals, in which, on day 33 of life, the number of active T-lymphocytes exceeded that of the control group by 7.0% ($P < 0.01$), the number of theophylline-resistant T-cells was 7.1% higher ($P < 0.01$), and the immunoregulatory index was 2.1 times higher ($P < 0.01$). At the same time, a 3.6% increase in the number of B-lymphocytes was recorded ($P < 0.05$), indicating an enhancement of the humoral immune response.

The most pronounced changes were observed in piglets in the group E2, which were fed 0.2 kg/t of AppetitX, confirming the dose-dependent nature of the immunomodulatory effect of the feed additive under investigation. The results obtained indicate the potential for using multi-component phytogenic compositions as a means of enhancing the immune potential and vitality of piglets without the use of antibiotic growth promoters.

Prospects for further research include investigating the effect of the essential oil composition on the functional activity of T- and B-lymphocytes, the production of cytokines and immunoglobulins, as well as assessing its efficacy during the post-weaning period, which is a critical stage in the development of immune resistance in piglets.

The authors state that they have no conflict of interests.

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