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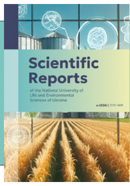

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The effect of a phytogenic preparation on productivity, biochemical blood status, and intestinal microbiota of replacement gilts

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Abstract. With the increasing use of antibiotics in pig farming, there is a growing need for alternative solutions to support animal health and productivity, which underscores the importance of studying the effectiveness of phytogenic additives in pig feeding. The study is dedicated to evaluating the impact of a phytogenic feed additive based on dried garlic and caraway on productivity, metabolic status, and the microbiota of the large intestine of replacement gilts of the (LW × L) genotype. The experiment was conducted under production conditions on two groups of animals (n = 80), which received either the basic diet without the additive (control) or the feed additive at a dose of 0.10% of the compound feed mass (experimental group). Starting from the 17th week of growth, the experimental group of gilts showed a significant advantage in live weight ($p \leqslant 0.001$) and average daily gains ($p \leqslant 0.01$), which persisted until the 28th week of age. The overall animal survival rate was 95% compared to 85% in the control group, indicating an improvement in physiological condition and stress resistance. Biochemical serum indicators showed an increase in albumin content, protein coefficient, calcium, phosphorus, and iron, while a decrease in γ-glutamyltransferase and indirect bilirubin was observed, reflecting optimisation of protein, mineral, and pigment metabolism, and a hepatoprotective effect. Bacteriological analysis revealed an increase in the proportion of *Lactobacillus amylovorus, L. reuteri*, *Clostridium butyricum*, and *Eubacterium rectale*, and a decrease in *Staphylococcus aureus, S. epidermidis*, and *Streptococcus suis*, indicating restoration of microbial

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balance, increased production of short-chain fatty acids, and colonisation resistance of the intestine. It was established that the phytogenic additive used exhibits a combined stimulating effect, simultaneously influencing the microbiota, metabolism, and physiological adaptation of the animals

Keywords: biochemical blood indicators; immunomodulation; sustainable pig farming; large intestine; phytobiotic

Introduction

Modern pig farming faces the challenge of increasing production efficiency while the use of antibiotics and synthetic growth stimulants continues to rise. In the context of transitioning to antibiotic-free technologies, an important direction is the use of natural additives that promote stable growth and development of young animals, strengthen the immune system, and improve the animals' adaptive capabilities. One such innovative solution is the use of phytogenic feed additives, which contain biologically active plant-derived components with antimicrobial, antioxidant, and anti-inflammatory effects.

Modern pig farming sets high demands for production efficiency, biosecurity, and animal health, while simultaneously reducing the use of antibiotics and synthetic growth stimulants. J. Wang *et al.* (2024) conducted a literature review examining phytogenic feed additives as natural alternatives to antibiotics in animal husbandry. The authors analysed numerous publications demonstrating the effectiveness of phytogenic additives in improving animal health and productivity. They also highlighted the potential of these additives to reduce the use of synthetic growth stimulants and antibiotics in livestock farming. According to N.L. Bevz & V.Ya. Lykhach (2024), in the context of transitioning to antibiotic-free production technologies, actively supported at both the European Union level and in Ukraine, the use of natural products in pig feeding has become particularly important. In their studies, R. Faustov *et al.* (2022) proved that the introduction of phytogenic preparations into the diet of fattening pigs ensures stable growth and development of young animals, strengthens the immune system, and enhances the adaptive capabilities of the organism. As noted by J. Lee *et al.* (2024), one of the promising directions for such solutions is the use of phytogenic feed additives, or phytobiotics, which contain biologically active plant-derived compounds – essential oils, phenolic substances, flavonoids, terpenoids, alkaloids, and other components with antimicrobial, antioxidant, and anti-inflammatory effects.

In the works of V.O. Alieksieiev (2025), it was noted that interest in phytogenic feed additives for different animal species has significantly increased. Phytogenic preparations, as evidenced by the data of N. Xiao *et al.* (2025), have shown the ability to positively affect digestion, stimulate enzyme secretion, stabilise intestinal microbiota, improve nutrient absorption, regulate immune response, and reduce oxidative stress levels. According to S. Biswas & I.H. Kim (2023), the introduction of phytogenic components into pig feeding contributes to increased average daily weight gain, improved feed conversion, reduced frequency of gastrointestinal disorders, and maintenance of stable physiological condition. These effects are due to the presence of essential oils and extracts containing substances with pronounced biological properties – carvacrol, thymol, allicin, limonene, etc., which exhibit a wide range of antimicrobial activity against pathogenic bacteria and fungi.

As noted by T.Yu. Mykhailenko & M.Yu. Sychov (2022), among the variety of phytogenic additives, significant attention is drawn to products based on garlic (*Allium sativum* L.) and caraway (*Carum carvi* L.), which have a synergistic effect in normalising intestinal microbiota, stimulating digestion, activating enzymatic processes, and increasing the body's resistance. It has been proven that the inclusion of garlic extract or powder in the diet of piglets contributes to improved growth rates, reduced cholesterol levels in serum, increased antioxidant activity, and normalisation of glucose and urea levels. The addition of caraway, in turn, enhances the secretory activity of digestive glands, stimulates appetite, inhibits the growth of pathogenic microbiota, and promotes the colonisation of beneficial lactobacilli in the intestine. Despite the study by M. Rabelo-Ruiz *et al.* (2021) on the use of phytogenic additives in pig feeding, the issue of their effectiveness in replacement young stock remains insufficiently studied. Most scientific works focus on the weaning or fattening period, while data on the impact of such additives on gilts during the growing period until their first insemination is limited. This period is crucial as active growth processes, formation of the reproductive organs, and physiological maturity occur, requiring optimal support for metabolic processes and the immune system. The reaction of replacement gilts' bodies to phytogenic stimulants in terms of biochemical blood indicators and changes in the microbiota composition of the large intestine, which plays a key role in maintaining metabolic homeostasis, is also insufficiently explored.

M. Walter *et al.* (2025) highlighted an important modern trend of applying a comprehensive approach to evaluate the effectiveness of feed additives, which includes not only productivity indicators but also physiological, biochemical, and microbiological parameters. This approach aligns with the modern concept of "gut health" – an integrated understanding of digestive tract health as the foundation for effective growth and productivity of animals. Q. Tang *et al.* (2025) demonstrated that studying the relationship between microbiota composition, metabolic processes, and immune system status allows for the development of new approaches to feeding technologies aimed at preventing stress conditions and enhancing the body's natural resistance. V. Ivanov *et al.* (2023) expressed the opinion that conducting such studies in real farm conditions, where factors such as microclimate, housing technology, animal genetic traits, and practical feeding aspects affect the results, holds significant scientific value. This ensures practical relevance and increases the importance of the obtained results for production. Furthermore, the use of modern laboratory methods ensures the reliability and accuracy of the data, meeting current international standards for experimental work in veterinary science and livestock production technologies.

Therefore, the relevance lies in justifying the effectiveness of using a phytogenic feed additive in the feeding of replacement gilts, which contributes to the optimisation of metabolic processes, maintenance of stable microbiota, and increased productivity without the use of antibiotics.

The aim of the study was to determine the impact of phytobiotic on productivity, biochemical blood indicators, and intestinal microbiota of replacement gilts during the growing period until their first insemination.

Materials and Methods

The study was conducted in 2023-2025 under the conditions of the privately leased enterprise "Victoria" in the Bashtanka district of Mykolaiv region. A total of 160 clinically healthy replacement gilts of a crossbred combination of Large White × Landrace (LW × L) from the breeding company "PIC" (UK) were used in the experiment. At 11 weeks of age, animals with a live weight of 32.02-32.62 kg were divided into two groups of 80 individuals each based on the analog method. Throughout the experiment, all experimental gilts received standard basic diets. Prior to the start of the scientific and production experiment, an acclimatisation period lasting 7 days (from 11 to 12 weeks of age) was conducted. The control group (I) received the basic diet (BD) without any additional feed additives. The experimental group (II) received an additional natural growth stimulator, "Imunochasnyk", at a dose of 1,000 g/ton of compound feed from the 12th to the 28th week of growth, according to the manufacturer's recommendations. The animals were fed specialised compound feeds for replacement gilts of periods I and II, manufactured in the farm's own feed mill. The diets were formulated based on the feeding strategies adopted by the farm and the methodological recommendations of genetic and feed companies (PIC – genetic company, n.d.).

Composition of "Imunochasnyk" (per 100 g of product): dried garlic (*Allium sativum* L) – 95 g, caraway (*Carum carvi* L) – 5 g, manufactured by order of "Eagle Trading LLC" (Ukraine), manufacturer: Argano Organis LLC, Unit 2 Industrial Estate, Saharanpur (India). The feed additive "Imunochasnyk" is a plant-based growth stimulator based on essential oils. Its composition includes essential oils of garlic and caraway at concentrations according to the manufacturer's guidelines. The use of this additive helps to stabilise digestion processes, promote the formation of healthy intestinal microbiota, enhance growth intensity, and improve the functional activity of the animals' immune system.

To ensure the adequacy of the diets in terms of nutrient content, premixes produced by LLC "Tsekhave Ukraine" were used. The experimental feed additive was incorporated directly into the compound feed during its manufacture at the feed mill when formulating the respective diets. Replacement gilts were fed a complete diet at a rate of 2.52 kg of feed per head per day with the following nutritional value: crude protein content of 155.0 g/kg and metabolisable energy of 12.36 MJ/kg. The composition of 1 kg of the complete feed included the following ingredients (%): maize (35), wheat (21), barley (10), wheat bran (12), sunflower meal (10), soybean meal (7), Cehavit sow premix replacement (5). Feed was provided ad libitum. Watering of the experimental animals was carried out using nipple drinkers at a ratio of one drinker per 10 animals.

Housing conditions complied with production standards and technological regulations for the agro-industrial complex (Ministry of Agrarian Policy of Ukraine, 2005). Replacement pigs were housed in groups of 20 animals per pen with a concrete slatted floor, providing a floor area of 1.0-1.65 m^2 per animal. Veterinary and preventive measures for animals of all experimental groups were carried out according to a unified scheme approved at the farm. Microclimate parameters in the housing facility for the experimental animals were ensured by the operation of a negative ventilation system, which included an axial exhaust fan installed in the ceiling section of the building and air inlet valves located in the walls of the facility. Coordination of the ventilation system components was performed using microprocessor-controlled equipment, which ensured automatic maintenance of the specified microclimate parameters. Manure removal from the facility was carried out using a vacuum–gravity system of periodic operation, which involved manure channels located beneath the entire pen area and a network of pipelines for transporting manure effluent to manure collectors situated outside the livestock building. Feeding, watering, housing conditions, animal care, and veterinary and preventive measures during the experiment were conducted in compliance with the requirements of current European and national legislation of Ukraine on the welfare of farm animals during their housing (Order of the Ministry…, 2021).

The productive performance of replacement gilts during the growing period until first insemination was assessed using a set of indicators, including live weight (kg) and average daily gain (g). In addition, herd survival rate and the proportion of culled animals during the specified period were determined in accordance with generally accepted methodological approaches (Voloshchuk, 2014; Ibatulin & Zhukorskyi, 2017; Ladyka & Khmelnychiy, 2023). Biochemical blood analyses of replacement gilts from the experimental and control groups at the end of the growing period, at 190 days of age, were conducted to evaluate the functional status of the liver and kidneys, protein, carbohydrate and mineral metabolism, as well as enzymatic activity of the organism. Blood samples were collected in the morning before feeding by puncture of the jugular vein from five clinically healthy animals in each group. Blood samples were collected into tubes containing a clot activator, followed by centrifugation to obtain serum. Biochemical analyses were performed in the independent laboratory of LLC "Expert Centre 'Biolaits'" using an automatic biochemical analyser ERBA XL-640 (Erba, Czech Republic) and appropriate certified reagents. The obtained results were compared with reference (normative) values for pigs of the corresponding age (Voloshchuk, 2014; Ibatulin & Zhukorskyi, 2017; Ladyka & Khmelnychiy, 2023).

At the final stage of the scientific and production experiment, the effect of the feed additive "Imunochasnyk" on the quantitative and species composition of the microbiota of the large intestine of replacement gilts was investigated. Faeces from the experimental gilts served as the material for studying the microbial composition; samples were collected from 10 animals in each group onto a clean substrate, after which they were transferred into sterile plastic containers and sent to the independent laboratory of LLC "Expert Centre 'Biolaits'". Microbiological analysis of faecal samples was conducted for the quantitative and qualitative determination of microorganisms through their identification using MALDI-TOF MS. The experimental research protocol was approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine in accordance with Good Clinical Practice (GCP) requirements regarding the protection and humane treatment of experimental animals, No. 039/2025 dated 26 June 2025.

The obtained research results were processed using statistical methods (Kramarenko *et al.*, 2019) with the use of computer technology and the STATISTICA v.7.0 software package (StatSoft Inc., USA), in accordance with the methodological approaches described by I.I. Ibatulin & O.M. Zhukorskyi (2017) and V.I. Ladyka & L.M. Khmelnychiy (2023).

Results and Discussion

The study of productive performance of replacement gilts of the (LW × L) genotype showed that at the initial stages of rearing (11-12 weeks of age), the live weight of animals in both groups was almost identical (32.02-32.62 kg), indicating homogeneity of the initial experimental material (Table 1). During this period, no significant differences were observed between the control (I) and experimental (II) groups, and culling due to injuries or diseases was minimal (0-2.5%). Starting from the 17th week of rearing, a significant advantage of the experimental group in live weight was established (66.22 kg) compared with the control group (61.40 kg, $p \leq 0.001$). Accordingly, average daily gains in animals receiving the feed additive were significantly higher, amounting to 817.14 g versus 731.43 g in the control group ($p \leq 0.01$), which indicated a stimulating effect of the additive on the growth intensity of the young animals.

Table 1. *Productive traits of replacement gilts (n = 80), $\bar{X} \pm S_{\bar{x}}$*

Trait	Group / Age	I (control)	II (experimental)
11 weeks			
Live weight, kg		32.62 ± 0.248	32.02 ± 0.330
12 weeks			
Live weight, kg		36.80 ± 0.434	37.62 ± 0.404
Culling (injuries, diseases), %		0	2.5
17 weeks			
Live weight, kg		61.40 ± 0.412	66.22 ± 0.384***
Average daily gain, g		731.43 ± 4.562	817.14 ± 3.632**
Culling (injuries, diseases), %		5.0	2.5
22 weeks			
Live weight, kg		88.16 ± 0.282	95.22 ± 0.314***

Table 1. *Continued*

Trait	Group / Age	I (control)	II (experimental)
22 weeks			
Average daily gain, g		764.57 ± 4.082	828.57 ± 3.420***
Culling (injuries, diseases, other), %		7.9	0
26 weeks			
Live weight, kg		**108.20 ± 0.308**	**117.80 ± 0.296*** **
Average daily gain, g		715.71 ± 3.502	806.43 ± 2.786***
Culling (injuries, diseases, other), %		0	0
28 weeks			
Live weight, kg		118.64 ± 0.282	128.52 ± 0.226***
Average daily gain, g		745.71 ± 4.40	765.71 ± 3.30**
Culling (injuries, diseases, other), %		2.9	0
Survival rate, %		85.0	95.0

Note: ** – p ≤ 0.01; *** – p ≤ 0.001
Source: authors' development

At 22 weeks of age, the differences between the groups persisted and even intensified: the live weight of gilts in the experimental group reached 95.22 kg, which significantly exceeded the control by 7.06 kg (p ≤ 0.001). Average daily gain during this period was also higher, amounting to 828.57 g compared with 764.57 g in the control group (p ≤ 0.001). No cases of culling were recorded in the experimental group, whereas in the control group it reached 7.9%, indicating a positive effect of the additive on animal health and viability. By 26 weeks of age, the advantage of the experimental group was maintained: the live weight of gilts reached 117.80 kg, which was 9.6 kg higher than in the control group (p ≤ 0.001). Average daily gain was also significantly higher (806.43 g versus 715.71 g, p ≤ 0.001). At this stage, no culling of animals was observed in either group. At the end of the growing period (28 weeks), the difference between the groups remained substantial. The live weight of gilts in the experimental group reached 128.52 kg, exceeding the control value (118.64 kg) by 9.88 kg (p ≤ 0.001). Average daily gains remained high, amounting to 765.71 g compared with 745.71 g in the control group (p ≤ 0.01). Overall survival in the experimental group was 95.0%, whereas in the control group it was 85.0%, confirming an improvement in physiological status and a reduction in stock losses due to the use of the investigated phytogenic feed additive. Thus, inclusion of the additive in the diet of replacement gilts at a dose of 0.10% of feed weight ensured increased growth intensity, improved average daily gains, and higher survival rates. This indicates its effectiveness as an immunostimulatory and growth-promoting agent in the system of rearing replacement pigs.

Biochemical indicators of blood serum reflect the state of metabolism, the functional activity of organs, and the response of the animal organism to dietary factors. Analysis of the obtained data demonstrated that pigs in the experimental group receiving the phytogenic feed additive exhibited positive changes in a number of metabolic indicators compared with the control group (Table 2).

Table 2. *Biochemical blood indicators of replacement gilts when using the phytogenic feed additive (n = 5),* $\bar{X} \pm S_{\bar{x}}$

Indicator	Reference range	Group		Difference II vs I
		I (control)	II (experimental)	
Albumins (ALB), g/L	25-45	22.34 ± 0.821	25.33 ± 0.621	2.99*
Alkaline phosphatase (ALP), U/L	30-150	258.37 ± 21.533	280.23 ± 20.819	21.86

***Table 2.** Continued*

Indicator	Reference range	Group		Difference II vs I
		I (control)	II (experimental)	
Alanine aminotransferase (ALT), U/L	12-54	63.82 ± 3.961	69.76 ± 8.471	5.94
Alpha-amylase (AMY), U/L	≤3500	2766.97 ± 351.098	3353.01 ± 399.331	586.04
Aspartate aminotransferase (AST), U/L	12-42	51.80 ± 4.754	66.19 ± 10.437	14.39
Calcium (Ca), mmol/L	2.0-3.0	2.41 ± 0.043	2.58 ± 0.035	0.17*
Cholesterol (CHOL), mmol/L	1.1-3.4	2.50 ± 0.063	2.56 ± 0.138	0.06
Creatinine (CRE), μmol/L	100-208	70.74 ± 3.992	83.54 ± 5.241	12.80
Direct bilirubin (DBIL), μmol/L	0-5.3	0.26 ± 0.063	0.27 ± 0.030	0.01
Gamma-glutamyltransferase (GGT), U/L	10-60	31.31 ± 5.210	25.84 ± 5.045	-5.47
Glucose (GLUC), mmol/L	2.5-8.1	5.25 ± 0.177	5.76 ± 0.363	0.51
Phosphorus (P), mmol/L	1.5-3.2	3.59 ± 0.122	3.70 ± 0.108	0.11
Iron (Fe), μmol/L	15-38	18.85 ± 1.643	28.72 ± 3.831	9.87*
Magnesium (Mg), mmol/L	0.74-1.32	0.89 ± 0.021	0.96 ± 0.028	0.07
Total bilirubin (TBIL), μmol/L	0.1-6.84	2.44 ± 0.090	2.33 ± 0.039	-0.11
Total protein (TP), g/L	50-85	55.81 ± 3.408	56.19 ± 2.201	0.38
Globulins (GLB), g/L	20-35	33.46 ± 3.569	30.86 ± 1.952	-2.60
Protein coefficient	0.8-1.2	0.72 ± 0.086	0.84 ± 0.051	0.12
De Ritis index (AST/ALT)	0.8-1.3	0.80 ± 0.032	0.92 ± 0.058	0.12
Ca/P ratio	1.2-1.8	0.68 ± 0.037	0.70 ± 0.032	0.02
Indirect bilirubin (IBIL), μmol/L	1.7-8.6	19.38 ± 1.429	8.94 ± 0.914	-10.44**

Note: ** – $p \leq 0.01$; *** – $p \leq 0.001$
Source: authors' development

Protein metabolism. The albumin content in the experimental group amounted to 25.33 g/L, which corresponded to the physiological range (25-45 g/L) and exceeded the control by 2.99 g/L. The values in the control group were below the lower limit of the norm. This indicated activation of protein synthesis in the liver and improvement of plastic processes. Total protein content in animals of both groups remained within the physiological range (50-85 g/L); however, a tendency towards a slight increase was observed in the experimental gilts (56.19 g/L versus 55.81 g/L). The globulin content in the experimental group decreased (30.86 g/L compared with 33.46 g/L in the control), which resulted in an increase in the protein coefficient from 0.72 to 0.84 – a value that reached the optimal range (0.8-1.2) in the experimental animals. This indicated a more balanced ratio between albumin and globulin synthesis, reflecting a stable immunobiochemical status.

Enzymatic activity. The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in pigs of both groups exceeded the reference ranges (12-54 and 12-42 U/L, respectively), which may be associated with intensive protein metabolism during the period of active growth. At the same time, in the experimental group the ALT level was higher (69.76 U/L), and AST exceeded the control by 14.39 U/L, which resulted in an increase in the de Ritis index to 0.92 (within the physiological range of 0.8-1.3). This indicated the absence of pathological changes in hepatocytes and good adaptation of the liver to the action of the phytogenic additive. Alkaline phosphatase (ALP) activity in the experimental group increased by 21.86 U/L, which was likely associated with intensified bone tissue growth processes. Gamma-glutamyltransferase (GGT) activity in animals of the experimental group decreased (25.84 versus 31.31 U/L), indicating a reduced hepatic load. Alpha-amylase activity remained within the normal range (up to 3,500 U/L) and showed a tendency to increase (3,353.01 U/L), which may indicate stimulation of pancreatic enzymatic activity.

Carbohydrate metabolism. The glucose concentration in the blood serum of pigs in both groups remained within the physiological range (2.5-8.1 mmol/L) and was slightly higher in the experimental animals (5.76 versus 5.25 mmol/L), which may be a consequence of more efficient nutrient utilisation when the experimental feed additive was included in the diet.

Mineral metabolism. The calcium and phosphorus contents in both groups were at adequate levels; however, in the experimental group an increase in calcium concentration to 2.58 mmol/L was observed, along with a slight increase in phosphorus to 3.70 mmol/L. Despite this, the Ca/P ratio remained below the optimal range (0.70 compared with the recommended 1.2-1.8), indicating a certain imbalance in mineral metabolism typical of young animals during periods of intensive growth. The magnesium content remained within the physiological range (0.96 mmol/L).

Pigment metabolism indicators. The levels of total and direct bilirubin remained within the physiological range, whereas the concentration of indirect bilirubin in the experimental group (8.94 μmol/L) decreased compared with the control group (19.38 μmol/L), indicating a reduction in haemolysis processes and stabilisation of the functional state of the liver.

Other metabolic indicators. Creatinine levels in animals of both groups were below the reference values (83.54 versus 70.74 μmol/L), which may be associated with age-related growth characteristics and a lower level of muscle metabolism compared with adult sows. The iron concentration in the experimental group significantly exceeded that of the control group (28.72 versus 18.85 μmol/L), indicating improved haematopoietic processes under the influence of the phytogenic additive. Cholesterol levels remained stable within the physiological range (2.56 mmol/L), indicating the absence of disturbances in lipid metabolism.

The obtained results demonstrated that the use of the phytogenic additive in the feeding of replacement gilts had a positive effect on protein, carbohydrate, and mineral metabolism, contributed to the stabilisation of hepatic enzymatic activity, and promoted improved protein synthesis and metabolic adaptation of the animals during the growing period.

Bacteriological analysis of samples from the large intestine of replacement gilts revealed the presence of a diverse commensal microbiota, represented mainly by Gram-positive cocci and rods of the genera *Streptococcus*, *Lactobacillus*, *Enterococcus*, and *Clostridium*. In the control group, facultative anaerobes predominated (*Streptococcus alactolyticus*, *Enterococcus faecalis*, *Staphylococcus* spp.), whereas in the experimental group acid-producing and butyrate-producing bacteria prevailed (*Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, *Eubacterium rectale*) (Table 3).

Table 3. *Changes in the species composition of the dominant microbiota of the large intestine of replacement gilts under the influence of the phytogenic feed additive, %*

Taxon	Group, n = 10 animals		Description
	I (control)	II (experimental)	
Streptococcus alactolyticus	100	100	Dominant commensal of the large intestine; ferments carbohydrates and maintains a stable pH environment.
Lactobacillus amylovorus	40	90	Lactic acid producer; competitively suppresses opportunistic microbiota.
Lactobacillus reuteri	30	70	Produces reuterin; reduces adhesion of *E. coli* and *Staphylococcus* spp.
Enterococcus faecalis	60	50	Permanent commensal; excessive levels are associated with inflammatory processes.
Staphylococcus aureus	40	10	Opportunistic pathogen; inhibited by phytogenic phenolic compounds.
Staphylococcus epidermidis	30	10	Reduced carriage indicates improved hygienic status of the intestinal mucosa.

Table 3. *Continued*

Taxon	Group, n = 10 animals		Description
	I (control)	II (experimental)	
Streptococcus suis	30	10	Potentially pathogenic; reduced carriage indicates a preventive effect.
Clostridium butyricum	20	40	Butyrate producer; improves epithelial regeneration and energy metabolism.
Eubacterium rectale	10	30	Produces butyrate; involved in stabilisation of the intestinal barrier function.
Escherichia coli (non-pathogenic strains)	30	15	Reduced putrefactive processes and proteolytic activity.

Source: authors' development

The presence of *Streptococcus alactolyticus* in 100% of samples from both groups confirms its role as a permanent dominant in the large intestine of pigs. As reported by H. Wang *et al.* (2023), *S. alactolyticus* contributes to carbohydrate fermentation and the production of short-chain fatty acids (SCFAs), which are essential for the energy supply of enterocytes. In the experimental group, the proportion of lactobacilli increased more than twofold. This may be attributed to the phytogenic additive containing garlic and caraway extracts, which likely stimulated the growth of lactic acid bacteria due to the presence of biologically active compounds with prebiotic properties. The observed reduction in the prevalence of *S. aureus*, *S. epidermidis*, and *S. suis* (by 3-4 times) may be associated with the antimicrobial activity of essential oils that disrupt the cell walls of Gram-positive bacteria. In addition, *Lactobacillus reuteri* produces reuterin, which exhibits bacteriostatic activity against *Staphylococcus* and *E. coli*, explaining the lower carriage rate of opportunistic microorganisms in the experimental group. *Clostridium butyricum* and *Eubacterium rectale* were detected in 40% and 30% of experimental samples, respectively, which was twice as high as in the control group. Butyrate is a key metabolite for nourishing the colonic epithelium and regulating apoptosis and anti-inflammatory responses. The increased abundance of these bacteria indicates activation of anaerobic fermentation and normalisation of microbial homeostasis.

The reduction in the prevalence of *Escherichia coli* in the experimental group to 15% reflects a positive effect of the phytogenic additive on the balance of the intestinal ecosystem. It is evident that an increased abundance of *Lactobacillus* spp. and butyrate-producing bacteria is associated with suppression of *E. coli* growth, a reduction in putrefactive processes, and improved nutrient utilisation. The complete repertoire of the presence/absence of microbiota taxa in intestinal samples from the experimental replacement gilts is presented in Figure 1. The obtained results demonstrated a classical pattern of microbial balance shift from facultative anaerobes towards obligate fermenters, which ensure a stable environment in the large intestine. This direction of change corresponds to contemporary concepts regarding the mechanism of action of phytogenic feed additives, namely stimulation of probiotic microbiota, inhibition of pathogenic microorganisms, and enhancement of intestinal colonisation resistance.

The obtained results of the study confirmed a significant positive effect of the experimental phytogenic feed additive on the productivity, metabolic status, and intestinal microbiota of replacement gilts. Starting from the 17th week of rearing, animals receiving the additive showed a significant advantage in live weight and average daily gains compared with the control group ($p \leq 0.001$), which persisted until the end of the experiment. At 28 weeks of age, animals in the experimental group demonstrated an average live weight of 128.52 kg compared with 118.64 kg in the control group, while average daily gains amounted to 765.71 g versus

745.71 g, respectively (p ⩽ 0.01). In addition, survival rate in the group of gilts receiving the experimental phytobiotic reached 95%, whereas in the control group this indicator did not exceed 85%. Similar results have been reported in contemporary scientific reviews. For example, M. Madesh *et al.* (2025) emphasised that the use of phytogenic feed additives in pig production contributes to improved productivity, nutrient utilisation, and feeding efficiency through modulation of the microbiota and stimulation of digestive enzymatic activity. Likewise, M. Mahboubi (2019) and H.A. Cho *et al.* (2024) demonstrated that inclusion of phytogenic mixtures in the diets of fattening pigs increased average daily gains and reduced stress-induced physiological responses. Thus, the results of the present experiment are consistent with current evidence indicating that phytogenic additives exert a combined stimulatory effect by simultaneously influencing the microbiota, metabolism, and overall physiological status of animals.

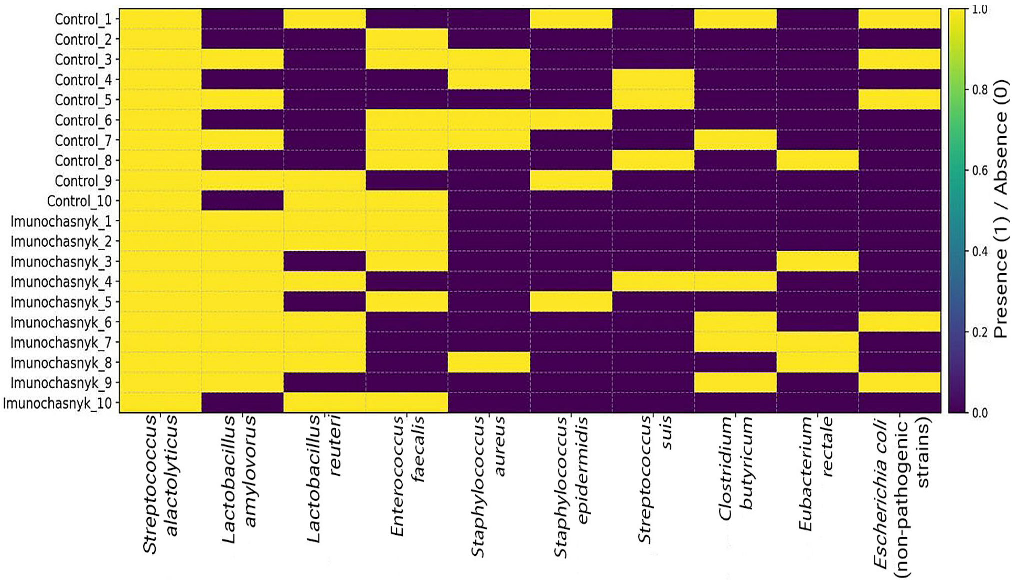

***Figure 1.** Presence/absence of species-level microbiota taxa in the experimental groups of replacement gilts*

Source: authors' development

Biochemical blood serum indicators reflected an improvement in metabolic processes and the functional state of the liver in replacement gilts receiving the experimental feed additive. An increase in albumin content (25.33 g/L) combined with a decrease in globulins resulted in an increase in the protein coefficient to 0.84, indicating optimisation of protein synthesis and a better balance between plastic and immune processes (Li *et al.*, 2021). In the present study, ALT and AST activities in animals of the experimental group exceeded those of the control group but remained within the physiological range, while the de Ritis index (0.92) indicated the absence of hepatocyte damage. An increase in alkaline phosphatase (ALP) activity by 21.86 U/L reflected intensification of growth processes and bone tissue remodelling, whereas a decrease in gamma-glutamyltransferase (GGT) to 25.84 U/L indicated a reduced hepatic load and improved detoxification function of the liver. Similar "liver-safe" profiles under phytogenic feeding strategies were described by F. Chen *et al.* (2024), who reported that phytogenic additives and essential oils in pigs improved antioxidant status without inducing pathological shifts in hepatic enzyme activity; under certain feeding regimens, an increase in plasma antioxidant enzymes was also observed without adverse effects on AST or ALT. Comparable trends were reported by A.O. John (2024) and V.G. Papatsiros *et al.* (2024), who documented improvements in haematological and enzymatic parameters in pigs receiving phytogenic feed additives.

The blood glucose concentration in the experimental animals (5.76 mmol/L) was slightly higher than in the control group (5.25 mmol/L), indicating more efficient carbohydrate utilisation and a more stable energy balance. Higher serum levels of calcium (2.58 mmol/L) and phosphorus (3.70 mmol/L) also reflected improved mineral metabolism and active growth processes. The increased iron concentration (28.72 versus 18.85 μmol/L) indicated stimulation of erythropoiesis and enhanced oxygen transport, thereby supporting more intensive growth of the animals. At the same time, the reduction in indirect bilirubin levels (8.94 versus 19.38 μmol/L in the control group) indicated stabilisation of liver functional status and a decrease in haemolysis processes. Similar changes were described by C.N. Shili *et al.* (2021), who emphasised that the state of the intestinal microbiota directly influences hepatoprotective function, hepatic enzyme activity, and overall metabolism in pigs. The biochemical results of the present study are consistent with the findings of E.R. Grela *et al.* (2013) and M. Mohammadi & I.H. Kim (2018) regarding the effects of phytogenic mixtures on oxidative stress and inflammatory markers in pigs. For example, supplementation of garlic powder combined with oregano essential oil in weaned piglets improved productivity without signs of systemic inflammation or adverse changes in oxidative stress biomarkers at an optimal dose (0.4%), whereas overdosing negated the beneficial effect, highlighting the importance of appropriate inclusion technology and dose titration. This aligns well with the concept of the present mixture at a dose of 0.1% as a "mild" modulator rather than an aggressive antimicrobial agent.

The microbiome shifts observed in the present study exhibited a clear functional vector: in the experimental group, the proportion of acid-producing and butyrate-generating taxa increased (in particular *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, and *Eubacterium rectale*), whereas in the control group facultative anaerobes predominated (*Streptococcus alactolyticus*, *Enterococcus faecalis*, *Staphylococcus* spp.). The direction of these changes fits well within the contemporary model of the "feed – microbiota – metabolism axis", whereby stimulation of microbial carbohydrate fermentation in the large intestine enhances the production of short-chain fatty acids (SCFAs), which nourish colonocytes, stabilise the intestinal barrier, and reduce systemic inflammation. Experimental studies by F. Wan *et al.* (2024) have demonstrated that phytogenic strategies and butyrate-oriented approaches modify the pig gut microbiome, increase SCFA production, and improve anti-inflammatory and antioxidant indices.

As shown by the results of the study, the application of *Lactobacillus reuteri*, particularly the LR1 strain, may serve as an effective means of improving intestinal barrier function in pigs. The high abundance of this strain in the experimental group indicates its role in modulating the microbiota of the large intestine, specifically by increasing the proportion of *Lactobacillus* spp., which has a beneficial effect on epithelial integrity. Similar findings have been reported in other studies, notably by B. Yang *et al.* (2022), who demonstrated that the use of *L. reuteri* LR1 improved the caecal microbiome and promoted restoration of intestinal barrier function in piglets. This effect may contribute to a reduction in the carriage of opportunistic pathogenic bacteria, such as enterobacteria and staphylococci, through the production of reuterin – a natural antimicrobial metabolite. In addition, the increased concentration of *Lactobacillus reuteri* in the experimental group of pigs confirms the ability of this strain to reduce adhesion of *Escherichia coli* and *Staphylococcus* spp., thereby improving the hygienic status of the intestinal mucosa and reducing the level of pathogenic microorganisms in the intestinal environment.

The increased proportion of butyrate-producing bacteria (*Clostridium butyricum*, *Eubacterium rectale*) provides a mechanistically convincing explanation for the biochemical shifts observed in the present study. Butyrate is the primary "fuel" for colonic enterocytes, modulates gene expression, exhibits anti-inflammatory properties, and is associated with improved metabolic efficiency. In pigs, enhancement of endogenous butyrate production through dietary approaches – such as supplementation with protected butyrate, prebiotics, or microbiome-targeted phytogenic mixtures – has been linked to reduced pro-inflammatory cytokine levels and improved antioxidant responses (Wan *et al.*, 2024). This is consistent with the ALP/GGT profile observed in the present study, as well as with improved indicators of energy metabolism (glucose), mineral metabolism (Ca, P), and erythropoiesis (Fe). In the current experiment, such effects may have been among the factors contributing to increased live weight and improved blood parameters, as also reported by J.O. Alagbe *et al.* (2024).

The role of garlic and caraway essential oils in the phytogenic feed additive also includes a selectively antimicrobial component: phenolic compounds (carvacrol and thymol), as well as sulphur-containing garlic compounds, particularly allicin, inhibit *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus suis* while not disrupting beneficial anaerobic consortia. Studies in pigs have already demonstrated that combinations of essential oils (oregano, mint, thyme, etc.) restructure the microbial profile in a direction favourable for productivity without compromising safety based on key biomarkers (Duarte & Kim, 2022). This provides additional support for the present observation of a reduced prevalence of *Escherichia coli* (from 30% to 15%) and a decline in staphylococci in the experimental group.

In summary, the positive changes in productivity and metabolic status observed in replacement gilts receiving the experimental phytobiotic can be attributed to the systemic effects of phytogenic components, which simultaneously regulate microbiota composition, enzymatic activity, protein and mineral metabolism, and the antioxidant status of the organism. This is consistent with the concept of the "feed – microbiota – metabolism axis", according to which a stable and balanced microbiota promotes more efficient energy utilisation, supports liver function, reduces systemic inflammation, and enhances animal productivity. Thus, the inclusion of the phytogenic additive "Imunochasnyk" in the diet of replacement gilts may be considered an effective means of biological modulation of metabolism, improvement of health status, and enhancement of growth intensity under industrial production conditions.

Conclusions

The results of the study confirm that the inclusion of a phytogenic feed additive in the diet of replacement gilts of the (LW × L) genotype at a dose of 0.10% of feed weight exerted a pronounced positive effect on productivity, metabolic status, and the microbiota of the large intestine. Starting from the 17th week of rearing, animals in the experimental group demonstrated a significant advantage in live weight and average daily gains ($p \leqslant 0.001$), which persisted until the end of the study; by 28 weeks of age, live weight exceeded the control by 9.88 kg, while herd survival reached 95%, which was 10% higher than in the control group. The experimental feed additive thus exhibited growth-promoting and preventive effects, improving the viability of replacement gilts. Biochemical indicators reflected optimisation of protein and mineral metabolism, increased albumin levels and protein coefficient, and reduced GGT and indirect bilirubin concentrations, indicating a hepatoprotective effect and improved metabolic adaptation. In addition, increased calcium, phosphorus, and iron levels indicated activation of bone formation and erythropoiesis, while elevated glucose concentration reflected a more stable energy balance.

Bacteriological investigations demonstrated that the use of the investigated feed additive induced a shift in the microbiota towards beneficial anaerobic microorganisms. In the experimental group, the proportions of *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum*, and *Eubacterium rectale* increased, whereas the abundance of potentially pathogenic *Staphylococcus* spp. and *Streptococcus suis* decreased by 3-4 times. Such microbial reconfiguration enhances the production of short-chain fatty acids, particularly butyrate, which improves intestinal barrier function and antioxidant status.

Overall, the experimental additive can be considered an effective and safe immunostimulatory agent for young pigs, exerting its effects through the "feed – microbiota – metabolism axis" mechanism and ensuring improved growth performance, maintenance of hepatic homeostasis, enhanced intestinal colonisation resistance, and better overall physiological status of the animals. Further studies should focus on determining the optimal dosage and duration of supplementation for different pig breeds, investigating the dynamics of immunological and antioxidant markers, and developing models of sustainable pig production based on the use of natural phytogenic stimulators.

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Conflict of Interest

None.

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Вплив фітогенного препарату на продуктивність, біохімічний статус крові та мікробіоту кишківника ремонтних свинок

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Анотація. В умовах збільшення використання антибіотиків у свинарстві зростає потреба в альтернативних рішеннях для підтримки здоров'я та продуктивності тварин, що обумовлює важливість дослідження ефективності фітогенних добавок у годівлі свиней. Дослідження присвячене оцінці впливу фітогенної кормової добавки, створеної на основі висушеного часнику та кмину, на продуктивність, метаболічний статус і мікробіоту товстого кишківника ремонтних свинок генотипу (ВБ × Л). Експеримент проведено у виробничих умовах на двох групах тварин (n = 80), які отримували основний раціон без добавки (контроль) або з додаванням кормової добавки у дозі 0,10 % від маси комбікорму (дослід). Починаючи з 17-го тижня вирощування, у свинок дослідної групи спостерігалося достовірне переважання за живою масою ($p \leqslant 0{,}001$) та середньодобовими приростами ($p \leqslant 0{,}01$), яке зберігалося до 28-тижневого віку. Загальна збереженість тварин становила 95 % проти 85 % у контролі, що свідчить про покращення фізіологічного стану та стійкості до стресу. Біохімічні показники сироватки крові засвідчили підвищення вмісту альбумінів, білкового коефіцієнта, кальцію, фосфору та заліза при одночасному зниженні γ-глутамілтрансферази і непрямого білірубіну, що відображає оптимізацію білкового, мінерального та пігментного обмінів і гепатопротекторний ефект. Бактеріологічний аналіз показав зростання частки *Lactobacillus amylovorus*, *L. reuteri*, *Clostridium butyricum* і *Eubacterium rectale* та зниження *Staphylococcus aureus*, *S. epidermidis* і *Streptococcus suis*, що свідчить про відновлення мікробного балансу, підвищення продукції коротколанцюгових жирних кислот і колонізаційної резистентності кишківника. Встановлено, що використана фітогенна добавка проявляє комбінований стимулюючий ефект, одночасно впливаючи на мікробіоту, обмін речовин і фізіологічну адаптацію тварин

Ключові слова: біохімічні показники крові; імуномодулювання; стале свинарство; товстий кишківник; фітобіотик