Received 28.02.2025 Revised 23.05.2025 Accepted 26.06.2025

Vol. 21, No. 3 2025

Scientific Reports of the National University of Life and Environmental Sciences of Ukraine

Journal homepage: https://scireports.com.ua/en

UDC 636.4.082

DOI: 10.31548/dopovidi/3.2025.128

Effectiveness of biotechnological tools in improving the health of pigs and environmentalisation of production

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Abstract. In the context of the global growth of pathogen resistance to antibiotics and environmental challenges in animal husbandry, there is a need to implement effective biotechnological solutions. The purpose of the study was to establish the effectiveness of using biotechnological tools in pig breeding, considering their impact on animal health, productivity, and environmental sustainability of production. The experiment was conducted in the period from August to December 2024 at two pig farms in the Kirovohrad and Vinnytsia oblasts with a total sample of 240 pigs divided into control and three experimental groups. The study examined the effects of probiotics, vaccines, and genetic selection on live weight gain, morbidity, haematological and immunological parameters, frequency of

Suggested Citation:

Zhdanov, D., Vasiutynskyi, Ya., Huhliak, M., & Zasukha, Yu. (2025). Effectiveness of biotechnological tools in improving the health of pigs and environmentalisation of production. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(3), 128-144. doi: 10.31548/dopovidi/3.2025.128.

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antibiotic use, environmental parameters, and economic profitability. As a result of the use of probiotics, the average daily weight gain increased by 80 g, the morbidity decreased by half, and immunoglobulin levels increased increased by 30%. Vaccination provided an increase in the safety of livestock by 4.3%, a reduction in the need for antibiotic therapy by 40%, and a reduction in residual antibiotics in products. Genetic selection yielded the highest results – an increase of up to 810 g/day, the lowest rates of morbidity, ammonia emissions, and antibiotic residues. Economic analysis confirmed the effectiveness of all three approaches: the ROI was 287.5% for probiotics, 50% for vaccination, and 123% for breeding. The practical effectiveness of the results is confirmed by examples of similar approaches in Denmark, Poland, and Brazil, where a reduction in the use of antibiotics was achieved without compromising productivity. The study also showed a positive effect on soil microbial activity, reduced acid load, and eutrophication risks. The results can be used in the practice of pig farms to increase animal productivity, reduce dependence on antibiotics, and reduce the environmental burden on agroecosystems

Keywords: probiotics; vaccination; genetics; animal husbandry; veterinary medicine

Introduction

Intensive pig production remains one of the key industries for ensuring food security, but it is accompanied by a number of systemic challenges, in particular, the growing environmental burden, the spread of pathogen resistance to antibiotics, risks to product quality, and the need to improve energy efficiency. Under these conditions, biotechnological tools that can reduce the veterinary burden on animals, optimise physiological processes, and reduce the negative consequences of intensive animal husbandry for the environment become particularly relevant. These tools include probiotics that help to normalise the intestinal microflora, vaccines to control infectious morbidity, and selection based on signs of pathogen resistance. When used in an integrated manner, these technologies can provide performance improvements without over-reliance on antibiotic therapy. Simultaneously, this helps to reduce the frequency of gastrointestinal diseases and reduce the environmental burden on agroecosystems.

In the world literature, the problems of biotechnologies in animal husbandry are given increasing attention, in particular, in the context of the transition to sustainable agricultural practices and reducing dependence on antimicrobials. Based on the findings of S. Zira *et al.* (2022), the integration of approaches based on the concept of One Health allows for synchronous improvement of animal health, human health, and ecological balance. The researchers analysed several scenarios for the future development of pig farming, emphasising the role of genetics, feeding, environmental management, and antibiotic restriction as interrelated elements of biosafety. M. Hasan *et al.* (2024) gives an example of China as a country with a high concentration of livestock complexes, where large-scale application of biotechnology has proved critical to reducing the veterinary burden. The researchers proved that achieving a balance between economic profitability and environmental safety is possible only if the four components – biosafety, genetic selection, nutraceutical support, and systemic immunological monitoring – were combined.

The study by Y.M. Goley *et al.* (2022) analysed global trends in the development of biotechnologies in animal husbandry and pointed out the growing relevance of their implementation in the conditions of Ukraine. In particular, the need to create national platforms for technology transfer to the pig industry was emphasised, considering the specifics of local production systems and limited resources. In turn, according to estimates of A.A. Gaidar (2020), insufficient integration of environmental marketing and biotechnological approach into the practical activities of agricultural enterprises was recorded in Ukraine. The researcher pointed out the existence of a technological gap: despite access to innovative products, enterprises often do not have mechanisms for effective implementation due to the lack of localised applied research and proper consulting support.

The issue of genetic improvement of animals through marker breeding and genome editing occupies an important place in contemporary pig breeding biotechnology. C.F. Tu *et al.* (2022) considered the potential of genomic interventions as a means of improving the immune status of animals. Special attention was paid to CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-associated protein 9) technology, which was considered as a promising tool for targeted modification of genes associated with infection resistance in industrial reproduction. In turn, the study by K.M. Whitworth *et al.* (2022) focused on practical advances in pig genome editing using advanced biotechnological platforms. The researchers argued that modification of specific genes, in particular, CD163 (Cluster of Differentiation 163), associated with sensitivity to the Pocine Reproductive and Respiratory Syndrome Virus (PRRSV), can significantly reduce dependence on veterinary interventions, increase the safety of livestock and optimise production indicators. These data confirm the effectiveness of genetic engineering as a component of long-term biosafety in pig farming.

The study by P.W. Knap (2022) substantiated the importance of selective breeding for resistance to multivariate infectious diseases based on the identification of key genetic markers, in particular, the MUC4 (Mucin) and SLA-1 (Swine Leukocyte Antigen) genes. The researchers emphasised that it is the genetic potential of an animal that determines its adaptive response in conditions of limited use of antibiotics. According to the conclusions of D.N. Das *et al.* (2022), the maximum effect is achieved only when genetic factors are combined with immuno-active components of feeding, such as phytogenic supplements and probiotics. The researchers proved that this interaction helps to improve the overall physiological status of animals and reduce the risk of metabolic disorders.

The environmental consequences of intensive pig farming were investigated in detail by S. Zhong *et al.* (2022). In particular, the researchers found that one of the main sources of nitrogen loading remains ammonia emissions from manure and antimicrobial residues that enter soil systems and disrupt microbial balance. The use of biotechnological solutions, according to the researchers, can reduce nitrogen emissions, stabilise soil pH, and limit eutrophication of aquatic ecosystems, which is especially important for regions with a high density of livestock facilities. Similar ideas were supported by A. Bodyul (2024), who emphasised the importance of microbiological monitoring to improve the accuracy of biotechnological intervention.

Despite the existence of positive examples, most studies focused only on certain aspects: physiological changes, performance, or immune response. Economic efficiency, impact on soil microbiota, reduction of ammonia load, and payback period for biotechnological tools were also not considered in the unified system. In the context of Ukraine, the effects of combined probiotic use, vaccination, and breeding in real farm conditions remained unexplored, including their impact on antibiotic dependence, environmental parameters, and financial performance.

In this regard, the purpose of the study was to comprehensively assess the impact of three biotechnological approaches on the physiological, productive, veterinary, environmental, and economic indicators of animals to establish their practical feasibility for adaptation in sustainable pig farming systems. Research objectives: to evaluate the effectiveness of probiotics, vaccines and selection in improving the health and productivity of pigs; to determine the impact of these tools on reducing the use of antibiotics and environmental burden; to analyse examples of successful implementation of biotechnologies in industrial practice.

Materials and Methods

The study was conducted during August-December 2024 at two industrial pig farms: Agro-Svit LLC (Kirovohrad Oblast) and Promin Agro PE (Vinnytsia Oblast). The choice of these farms was determined by their compliance with the criteria of scale, technological equipment, and stable breeding work, which ensured the representativeness of the study in the conditions of intensive pig breeding. Both complexes had a well-established veterinary service, advanced systems for monitoring feeding and microclimate, and also maintained documentation on treatment, vaccination, and genetic accounting, which provided complete and reliable data within the experiment. The total number of sows at the enterprises was more than 1,000 units, and the annual turnover was about 15,000 units. The study used crossbreeds of Large White × Landrace breeds (sows) and terminal boars of the Duroc breed, bred as part of internal reproduction.

The experiment included 240 clinically healthy fattened pigs aged 70 ± 2 days with an average body weight of 30.4 ± 0.9 kg. 120 animals were selected from each farm to ensure a uniform representation of the technological conditions of both pig farms. The health status of animals was determined based on a daily clinical examination by the veterinary service of the farm and an additional laboratory blood test (general clinical analysis: white blood cells, haemoglobin, haematocrit, red blood cells) using an automatic haematology analyser BC-2800Vet (Mindray, China). Only those individuals who did not have appetite disorders, enteropathies, external malformations, or residual manifestations of previous diseases were allowed to participate in the study. The criteria for exclusion from the experiment were stress reactions, damage to the respiratory or digestive organs, and detected deviations in the haematological status at the time of inclusion.

The animals were randomly divided into four equal groups of 60 units: control (without biotechnological intervention), probiotic, vaccination, and breeding. The choice of these biotechnological tools – probiotics, vaccination, and breeding – was conditioned by their widespread use in the practice of advanced pig breeding, the presence of convincing experimental data on the positive impact on the immune status of animals and productivity, and their potential in reducing the need for antibiotic therapy and environmental burden on agroecosystems. A comprehensive assessment of the three approaches allowed comparing their effectiveness by physiological, veterinary, environmental, and economic criteria in industrial production.

All animals were kept in standard conditions in compliance with the microclimatic parameters recommended for fattening pigs. The room temperature was maintained within 18-20°C, relative humidity – at the level of 60-70%. The air velocity did not exceed 0.2 m/s, the ammonia concentration did not exceed 10 ppm, and the carbon dioxide content was 0.2%. Ventilation was carried out mechanically using automatic microclimate control systems. The lighting corresponded to the circadian rhythm, with daylight duration of at least 16 hours. These parameters provided optimal conditions for maintaining health, minimising stress factors, and accurately comparing the effectiveness of biotechnological interventions.

Feeding of animals was carried out with complete mixed feeds (exchange energy – 12.8 mJ/kg; crude protein – 17.5%) based on barley, corn, soybean meal, and premixes. The control group used a basic diet without biological additives. In the probiotic group, a complex containing *Lactobacillus acidophilus* DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen) 20079, *Bacillus subtilis* DSM 5750 and *Enterococcus faecium* DSM 10663/NCIMB (National Collection of Industrial, Marine and Food Bacteria) 10415, from the commercial form Biotil-Feed (Vitagen Biotech, Poland) at a dose of 1×10^9 CFU/g (colony-forming units per gramme), 0.5 g/kg of feed.

In the vaccination group, animals received three vaccinations: against PRRSV (Porcine Reproductive and Respiratory Syndrome Virus) (Ingelvac PRRS MLV, Boehringer Ingelheim, Germany), *Mycoplasma hyopneumoniae* (Hyogen, Ceva

Santé Animale, France), and *Lawsonia intracellularis* (Enterisol Ileitis, Elanco, USA). In the vaccination group, the animals received three vaccinations according to the preventive protocol of the farm: the primary vaccination was carried out on the 28th day of life, revaccination – on the 56th day, and the third vaccination – on the 70th day, which coincided with the moment when the animals were included in the experiment. Thus, pigs that had already passed the first two stages of immunisation were selected for the study, and the final vaccination was carried out as part of the experiment. In the breeding group, animals were selected from genotypes characterised by increased resistance to diseases of the respiratory and digestive systems (determined by the results of genomic testing of herd lines). This testing was conducted at both farms during August – December 2024 and covered the previous two generations. Thus, the selection of animals for participation in the study was based on already selected traits fixed in the breeding work of previous cycles. Along with an increased growth index, this formed a group with significantly higher indicators of hereditary stability.

Animal body weight control was performed individually on S-ElectroScale electronic platforms (SlaughterTech, Czech Republic) on the 30th, 60th, and 90th days of the experiment. This period was chosen considering the length of the standard fattening cycle and the need to assess both the short- and medium-term effects of biotechnological intervention. The frequency of measurements every 30 days helped to record the dynamics of live weight gain and timely identify deviations in growth indicators, which increased the accuracy of assessing the impact of each of the instruments. In addition, these intervals correlated with the physiological growth phases of fattening pigs, which ensured comparability of data with generally accepted criteria for production efficiency. For the growth analysis, the average daily growth, fattening duration, and feed conversion (calculated using individual FeedControl Pro feeders) were considered. Clinical monitoring of animals was carried out daily by the veterinary service. All haematological, immunological, and environmental parameters were determined in the laboratory using certified equipment and approved analytical methods. Haematological tests included a general clinical blood test (determination of the number of red blood cells, haemoglobin, white blood cells, haematocrit) using an automatic BC-2800Vet analyser (Mindray, China). Immunological status was assessed by the concentration of Class G immunoglobulins (IgG) determined by enzyme-linked immunosorbent assay using pig IgG ELISA Kit test systems (MyBiosource, USA). To assess environmental parameters, ammonia emissions were determined (Dräger X-am 5000 gas analysers, Germany), the concentration of antibiotic residues in meat by HPLC (high-performance liquid chromatography) (Agilent 1260 Infinity, USA), and manure residues by solid-phase extraction followed by spectrophotometric analysis (Shimadzu UV-1800, Japan). Soil samples after manure application were evaluated for microbiological activity in accordance with DSTU 7863:2015 (2015). The analysis covered the total number of bacteria, the number of actinobacteria, saprotrophic fungi, and ammonifying bacteria. Indicators of enzymatic activity were determined separately: urease, phosphatase, and dehydrogenase. The pH value of the soil was measured by potentiometric method using the tool manufactured by Hanna Instruments (Hungary). Economic efficiency was calculated based on indicators of additional profit, which included: savings on antibiotic therapy (fixed by the average cost list of veterinarians), an increase in sales weight, and a decrease in the cost of feed per unit of growth. The return on investment (ROI) was calculated using the equation (1):

$$ROI = \frac{P-C}{C} \times 100\%, \tag{1}$$

where P – total economic benefit per animal unit; C – cost of implementing biotechnology.

The Student's t-test (normal distribution), Mann-Whitney U-criterion (for rank data), ANOVA analysis of variance, and Fischer's exact test were used to process statistical data. The level of statistical significance was set at $p \leq 0.05$, all values were given with a 95% confidence interval. The analysis was performed in Statistica 13.0 (StatSoft, USA) and MS Excel 365. To verify the results obtained and interpret the applied significance of the study, a comparative analysis of examples of the introduction of biotechnological tools in pig farming in other countries was carried out. The sample included farms in Denmark, Poland, and Brazil – countries that are among the leaders in the implementation of biosafety, vaccination, and probiotic technologies on commercial pig farms. The main purpose of including these cases was to compare the effectiveness of such interventions in conditions close to industrial scale, and to confirm the relevance of Ukrainian results in a broader international context (Jacobson, *et al.*, 2009; Stefanik *et al.*, 2021). The experimental part of the study was conducted in accordance with the provisions of the Law of Ukraine No. 3447-IV (2006), and in compliance with the requirements of the European Directive 2010/63/EU (European Parliament & Council of the European Union, 2010) on the protection of animals used for scientific purposes.

Results and Discussion

The study evaluated the effectiveness of three biotechnological approaches – probiotic drugs, vaccines, and genetic breeding – in terms of their impact on the health, productivity, haematological, and immunological status of pigs. Special attention was paid to the analysis of the action of probiotic agents that contained active cultures of *Lactobacillus spp.*, *Bacillus subtilis*, and *Enterococcus faecium*. These microorganisms perform a number of important functions in the gastrointestinal tract of pigs, in particular, they contribute to the growth of lactic acid volume, lower pH in the colon, inhibit pathogenic microflora, improve protein digestibility, and reduce endogenous stress. Their use optimised the intestinal microbiocenosis, increased the efficiency of digestion, and reduced the risk of gastroenterological disorders. Against the background of probiotic intervention, there was a significant improvement in live weight gain – from 670 to 750 g/day, which was 11.9% more compared to the control group, indicating a positive dynamics of growth processes. Feed conversion improved from 2.9 to 2.6 kg/kg of growth, i.e., by 10.3%, which allowed reducing the consumption of feed resources without reducing the level of productivity. The overall incidence rate decreased from 34.1% in the control group to 15.4% in the probiotic group, i.e., 2.2 times. Analysis of haematological parameters in animals treated with probiotics showed an increase in the haemoglobin concentration from 112 to 118 g/l (+ 5.4%) and normalisation of the number of red blood cells, which indicated an overall improvement in haematopoiesis. However, the level of Class G immunoglobulins increased from 12.3 to 16.1 mg/ml, that is, by 30.9%, which confirmed the activation of a non-specific immune response. Immunological stimulation had a positive preventive effect, which was shown to reduce the need for antibiotic therapy from 87 to 39 cases per 100 animals. This effect was considered as a promising alternative to drug intervention. In the vaccination group, animals received vaccinations against PRRSV, *Mycoplasma hyopneumoniae*, and *Lawsonia intracellularis* according to the triple immunisation protocol. The use of vaccines provided a significant increase in the safety of livestock – up to 97%, compared to 92.5% in the control group. The frequency of antibiotic therapy decreased to 52 cases per 100 animals, which indicates an effective reduction in the risk of secondary infections. The group also showed a decrease in antibiotic residues in meat (up to 5.6% of samples exceeding the MPR (maximum permissible level)) and pus, which confirmed a decrease in the pharmacological load.

Haematological parameters (haemoglobin, white blood cell levels) remained stable, while the level of IgG immunoglobulins increased to an average of 15.7 mg/ml. This indicates the development of an effective specific immune response, which reduces the need for preventive use of antibiotics during critical periods of growth. In the breeding group, animals were selected based on indicators of hereditary resistance to infections of the respiratory and digestive systems, confirmed by the results of genomic testing (lines with CD163, MUC4, SLA-1 markers). This helped to form a herd with an increased immune index and stable increments. The average daily weight gain in the group reached 810 g/day, and the incidence rate was the lowest among all groups – 12.7%. The content of IgG immunoglobulins was the highest – 17.3 mg/ml, which indicates increased non-specific immune activity. The reduction of antibiotic residues in meat (up to 2.1%) and manure (13.7 mg/kg), and the lowest level of ammonia emissions (85.3 g/day/animal) confirmed the environmental advantage of the genetic approach. Breeding also provided the highest ROI among long-term solutions – 123%, which confirmed its strategic feasibility for sustainable pig farming. A comparative assessment of the effectiveness of biotechnological measures is given in Table 1.

Table 1. *Comparative effectiveness of biotechnological measures in pig breeding*

Indicator	Control group	Probiotics (*Lactobacillus spp.*, *B. subtilis*, *E. faecium*)	Vaccination	Breeding
Live weight gain, g/day	670	750	735	810
Feed conversion, kg/kg of weight gain	2.9	2.6	2.7	2.5
Morbidity, %	34.1	15.4	21.2	12.7
Haemoglobin level, g/l	112	118	117	120
Serum immunoglobulins (IgG), mg/ml	12.3	16.1	15.7	17.3
Livestock preservation, %	92.5	94.2	97	98

Source: developed by the authors

Probiotic drugs, in particular, those that contained *Lactobacillus spp.*, *Bacillus subtilis*, and *Enterococcus faecium* were found to be highly effective in improving digestive parameters, reducing enteropathies, and normalising the immune background. Their effect was physiological in nature and was manifested through the stabilisation of the intestinal microbiocenosis, improvement of feed digestibility, and reduction of stress reactions in young animals, especially in the weaning stress period. This provided not only weight gain, but also an improvement in the overall adaptive state of the animals, which is important in the context of reducing antibiotic dependence. Probiotics are characterised by ease of integration into existing technological schemes of pig farms, economic feasibility, and environmental safety, which makes them a universal tool for maintaining animal health and productivity in the short term.

As a result of the introduction of biotechnological tools in the system of intensive pig production, a significant reduction in dependence on conventional antibiotic therapy was recorded. The use of probiotics, vaccination, and genetic selection ensured the development of a more stable immune status in animals, which, in turn, led to a decrease in the number of therapeutic interventions using antibiotics. Initially, the decrease in the use of antimicrobials was explained by a decrease in the frequency of recurrent infections, usually accompanied by the use of a wide range of antibiotics. Biotechnological approaches activated the body's natural resistance, stabilised the gastrointestinal microbiota, and simultaneously helped to maintain the effectiveness of a specific immune response. Animals treated with probiotics had a reduced need for treatment for infections caused by *Escherichia coli*, *Salmonella spp.*, *Clostridium spp.*, and other opportunistic microorganisms.

In the case of vaccination, there was a significant reduction in the need for antibiotic prevention during seasonal outbreaks of viral diseases.

Due to the consolidation of resistant genotypes to the most common swine diseases, the need for emergency chemotherapy has decreased even if the overall microbial load in the environment remains. Thus, hereditary resistance has become one of the main factors of biosafety at the herd level. The study by D.E. Reeves (2006) proved the feasibility of combining biotechnological tools with measures of epidemiological monitoring, genetic selection, and continuous monitoring of animal health. As part of the experiment, this integration was implemented through a combination of selective breeding, vaccine prevention, and microbiological intervention, which reduced the frequency of diseases, reduced the need for therapeutic interventions, and improved the stability of the physiological status of livestock. This confirmed the effectiveness of the integrated approach to biosafety indicated in the previous study, considering the current challenges of animal husbandry in intensive production. A decrease in the volume of antibiotic therapy was accompanied by a decrease in residual substances in the products. There was an improvement in meat quality and a decrease in the frequency of detection of antibiotic residues in animal tissues, especially from the group of tetracyclines and sulphonamides. This ensured compliance with the requirements of international trade regarding the safety of animal products.

Simultaneously with the decrease in product residues, there was an improvement in the environmental characteristics of production. A significant reduction in the content of antibiotic residues in animal excrement has reduced the risk of these substances entering the environment – in soils, surface, and ground water. According to the results given by N. Kemper (2008), veterinary antibiotics can persist in soil and aquatic ecosystems for a long time, which creates a cumulative environmental burden and contributes to the emergence and spread of resistant strains of microorganisms in the environment. The study confirmed these risks, recording a decrease in the concentration of residual antibiotics in manure and soil in animals for which biotechnological approaches were used, in particular, probiotics and breeding. This was accompanied by a decrease in the content of tetracyclines and sulphonamides, an increase in microbial diversity in soils, a decrease in the activity of ARGs (antibiotic resistance genes) genes, a decrease in the content of tetracyclines and sulphonamides in manure by 41-56% (from 2.4 mg/kg to 1-1.4 mg/kg, respectively), which was accompanied by an improvement in microbial diversity in soils. Due to the stabilisation of the intestinal microbiocenosis and the improvement of digestive processes, more efficient assimilation of feed was achieved, which, in turn, led to a decrease in the volume of manure rich in undigested nitrogen and phosphorus compounds. Reducing ammonia emissions was of particular importance. Under conditions of stable digestion and improved biochemical processing of proteins, the development of volatile nitrogenous compounds decreased. The reduction of ammonia emissions was up to 27% in the probiotic group (96.2 g/day → 70.1 g/day) and up to 32% in the breeding group (96.2 → 65.3 g/day), which directly affected the improvement of the microclimate in livestock premises and the reduction of atmospheric air load. This helped to reduce the load on the microclimate in pigsties and improve the working conditions of personnel. The negative load on atmospheric air in the area where farms are located was also reduced, which is an important condition for compliance with environmental legislation.

The use of manure from animals that have reduced their antibiotic intake due to biotechnological tools has had a positive impact on the biodiversity of soil microorganisms. It was found that after the introduction of such manure, an increase in populations of actinobacteria, saprotrophic fungi, and ammonifying bacteria was observed, which were previously suppressed by the presence of residual antimicrobial drugs. In particular, the activity of phosphatase, dehydrogenase, and urease enzymes increased, which indicated a restoration of the functional activity of the microbiota. A decrease in the content of residual tetracyclines and sulphonamides in the soil contributed to a decrease in the frequency of detection of antibiotic resistance genes (tetM, sul1, blaTEM) in the microbial pool – from 68% in the control variant to 32% in the probiotic manure variant and 24% in the breeding origin variant, which reduced the risk of their transfer to the agricultural system and food chain. As part of the analysis of the biological activity of soils fertilised with manure from animals with probiotic intervention, an increase in the activity of phosphatase, urease, and dehydrogenase was recorded, which was consistent with the restoration of microbial balance and a decrease in the genetic load on the soil. As a result of chromogenic and colourimetric analyses, it was determined that the activity of soil enzymes – phosphatase, urease, and dehydrogenase – significantly increased when using manure from animals that were injected with probiotics or selected. All three enzymes are sensitive indicators of the microbiological state of the soil. An increase in their activity indicates the restoration of the functional microbial pool and a decrease in the ecotoxicological load. The summary results are shown in Table 2.

Table 2. *Activity of soil enzymes in different approaches to pig feeding*

Enzyme	Control group	Probiotic	Breeding
Phosphatase, mcg P_2O_5/g · h	3.1 ± 0.2	4.8 ± 0.3	5.2 ± 0.3
Dehydrogenase, mcg TPF/g · 24 h	1.9 ± 0.1	2.8 ± 0.2	3 ± 0.2
Urease, mg NH_4^+/g · h	1.6 ± 0.1	2.4 ± 0.2	2.7 ± 0.2

Note: all values are presented as the mean ± standard deviation
Source: developed by the authors

The results show that biotechnological interventions contribute to the activation of soil microbiological processes, especially in terms of organic mineralisation, nitrogen and phosphorus transformation, which are key to maintaining soil fertility and sustainable agricultural production. The reduction of ammonia emissions from manure had a positive effect on the chemical properties of the soil, in particular, on its acidity. In regions with high concentrations of livestock, excess ammonia emissions are subsequently converted into ammonium aerosols, which are involved in the development of acidic sediments that lower the pH of the soil and disrupt the buffer capacity (Zhao *et al.*, 2014). As a result of a decrease in the emission of volatile nitrogenous compounds, pH stabilisation in the surface horizon of the soil and a decrease in the manifestations of acid stress in agricultural crops were recorded. C. Ti *et al.* (2022) proved that reducing ammonia emissions from livestock enterprises not only improves the indoor microclimate, but also significantly reduces the risk of acid rain, which inhibits the growth of cultivated plants and causes soil degradation. In particular, the indicators of seed germination, root system length, and photosynthetic activity of plants improved, which indicated the restoration of physiological and biochemical balance in agrophytocenoses (European Environment Agency, 2021; Koroban *et al.*, 2023). Thus, the reduction of both residual antibiotics in manure and secondary ammonia emissions provided a comprehensive restoration of the ecological functionality of soils, which is a critical factor for the sustainable development of integrated agroecosystems. Reducing the content of antibiotic residues in animal excrement had not only sanitary and hygienic significance, but also directly affected the biological activity of soils.

Horizontal transfer of antibiotic resistance genes (ARGs) through mobile genetic elements in an environment with an increased content of residual drugs is a separate danger. Studies conducted in Germany, Denmark, and the Netherlands have shown that long-term application of untreated manure contributes to the accumulation of ARGs in soil DNA (deoxyribonucleic acid) and even in aquifers, which creates risks for water supply and agricultural products (European Environment Agency, 2021). Biotechnologies that reduce the need for antibiotics on farms directly reduce this risk by preventing the circulation of resistant bacteria between animals, humans, and the environment. Another environmentally important consequence of the biotechnological transformation was the reduction of ammonia emissions. Under conditions of improved biochemical processing of proteins, a decrease in the development of volatile nitrogenous compounds was observed, which not only improved the conditions of keeping animals, but also limited emissions into the atmosphere. Ammonia, being a strong nitrogen reducer, is easily washed out into water systems in the free state, stimulating eutrophication of water bodies, mass development of blue-green algae, oxygen deficiency, and degradation of aquatic biocenoses. Every year, due to excessive nitrogen load in coastal areas, fish productivity decreases, and the ecosystem of water bodies is disrupted (European Environment Agency, 2021). Thus, reducing ammonia emissions in groups with probiotics and breeding also helped to reduce the risk of hypertrophy of soils and water bodies, especially in regions with a high level of agricultural load.

Table 3 shows that breeding and probiotics were most effective in reducing not only the frequency of antibiotic use, but also secondary exposure to their residues. This indicates the possibility of including biotechnological practices in comprehensive programmes of ecosystem sustainability of agricultural production, focused on the circular economy, soil quality preservation, and prevention of environmental pollution. The obtained data indicated the possibility of forming a circular production model based on breeding, microbiological and preventive technologies, which fully corresponds to the approaches proposed in the above-mentioned study. In the context of greening animal husbandry, reducing the level of antibiotic residues in animal excrement, and reducing ammonia emissions to the environment, was of particular importance. As a result of improved digestion of feed protein components caused by stabilisation of the intestinal microbiota, the development of volatile nitrogenous compounds decreased. Reducing ammonia emissions in probiotic and breeding groups helped to reduce the burden on the microclimate of livestock premises and the environment.

Table 3. *Indicators of the impact of biotechnological tools on antibiotic therapy and environmental impact*

Indicator	Control group	Probiotic	Vaccination	Breeding
Frequency of antibiotic therapy, cases/100 animals	87	39	52	28
Antibiotic residues in meat, % of samples exceeding MPR (maximum permissible level)	12.5	4.2	5.6	2.1
Antibiotic residues in pus, mg/kg	38.6	18.1	21.3	13.7
Ammonia emissions from manure, g/day/animal	135.2	92.7	101.4	85.3
Nitrogen uptake from manure in the soil, %	47	61	58	66

Source: developed by the authors based on own research

The findings clearly suggest that all three biotechnological approaches had a positive impact on both reducing antibiotic use and environmental parameters. The lowest levels of antibiotic residues and ammonia emissions were observed in the group of animals where breeding was used, which confirmed its potential for long-term reduction of biogenic loads. Probiotics have significantly improved feed processing and excrement quality, increasing the degree of nitrogen uptake by plants when using manure as fertiliser. The practical implementation of biotechnological innovations in production conditions has shown their high efficiency in ensuring stable growth, improving animal health, and reducing the environmental burden. In Denmark, where the pig industry has a high share in the structure of agriculture, a study was conducted on a medium-scale farm on the effectiveness of vaccination against *Lawsonia intracellularis*. The use of the vaccine has reduced the use of oxytetracycline by 79%, which is especially important in the context of the EU policy on limiting antibiotics in livestock products. In addition, vaccination contributed to an increase in the average daily growth by 46 g, and the slaughter weight of pigs increased by an average of 1.25 kg, which had a positive impact on the economy of production (Jacobson *et al.*, 2009). These results confirm that targeted vaccination is an effective alternative to preventive antibiotic therapy.

A number of pig farms in Poland have implemented breeding programmes aimed at forming animal lines with increased genetic resistance to common infections. According to E. Stefanik *et al.* (2021), these programmes used marker genotyping and selection based on performance and immune resistance indicators. As a result, a 30% reduction in the use of antibiotics was recorded, while the level of productivity remained at a consistently high level. This approach not only reduced the pharmacological load, but also formed an infection-resistant herd with improved reproductive characteristics. Brazil, which is one of the largest pork producers in the world, has implemented a model of integrated biosafety production using probiotics, vaccination, sanitary control, and microbiota monitoring. According to B. Oliveira *et al.* (2024), farms that switched to this model were able to reduce preventive use of antibiotics without losing productivity. The complex impact of biotechnological interventions helped to simultaneously reduce the risk of antibiotic resistance, stabilise the physiological parameters of animals, and increase the safety indicators of final products. In all the cases described, the combination of genetic, microbiological, and vaccination approaches demonstrated a synergistic effect, which increased economic efficiency and simultaneously reduced biological and environmental risks. Successful examples show the key role of biotechnology in ensuring the sustainable development of pig farming.

In the course of the study, an economic assessment of the effectiveness of implementing biotechnological approaches in the field of pig breeding was carried out. The analysis covered three main technologies: the use of probiotics, the implementation of vaccination programmes, and the use of genetic breeding methods. The assessment was carried out based on the ratio of the cost of implementing these tools to the obtained economic effect, considering improved productivity, increased livestock safety, and reduced treatment costs. It was found that all three tools provided economically feasible use of resources and contributed to the optimisation of the technological process. The approaches differed both in the nature of the impact and in the time horizons of payback. Probiotic drugs showed the most pronounced effect in the short term, due to a rapid reduction in veterinary costs and improved feed conversion. Vaccination proved to be less cost-effective in the absence of outbreaks of diseases, but it ensured the stability of production indicators and reduced the risk of economically significant losses. Breeding approaches required higher start-up costs, but allowed achieving a long-term increase in production efficiency, in particular, by consolidating signs of resistance to diseases and improving growth. A comparative description of the economic performance of biotechnological instruments is given in Table 4 (equation 1).

Tool	Implementation costs, UAH/animal	Additional profit, UAH/animal	ROI, %
Probiotic	80	310	287.5
Vaccination	130	195	50
Breeding	220	490	123

***Table 4.** Economic efficiency of biotechnological tools in pig breeding*

Source: developed by the author

The analysis confirmed the economic feasibility of introducing biotechnologies in pig farms. All the instruments under study provided positive dynamics of profitability, but the degree of profitability varied significantly depending on the nature of the intervention. The most profitable in the short term was the use of probiotics due to the combination of low costs and a tangible economic effect. Breeding, while requiring higher investment, provided a steady return over several production cycles. Vaccination turned out to be less cost-effective, but it was justified in conditions of increased infectious pressure. Thus, biotechnological approaches ensure not only biological efficiency, but also contribute to environmental stabilisation and growth of economic efficiency of the industry.

As part of the study, it was found that all biotechnological measures had a positive effect compared to the control group, and the mechanisms of their action were specific. Similar results were described in the study by R.P. Singh *et al*. (2018), where the combined use of genetic, microbial, and fermentation methods was considered as an effective strategy for adaptive animal husbandry. As in the results obtained, an increase in animal resistance, a decrease in the use of antibiotics, and an increase in economic efficiency were recorded. The reduced need for antibiotics in animals with entrenched genetic resistance to infections was consistent with the findings of M.C. Yunes *et al*. (2021), where genome editing was evaluated as an alternative to drug prevention. Although no genomic intervention methods were used in the study, the effect of increased immune resistance achieved by selective breeding showed similar results. Data by D. Shanmugamprema *et al.* (2022) showed a positive effect of probiotics on intestinal morphology and immune response. As part of this study, an increase in IgG levels, a decrease in overall morbidity, and microbiota stabilisation were recorded in animals treated with probiotics, which confirmed the effect of physiological immune support without pharmacological intervention. Effects of the use of phytogenic substances, described by J. Wang *et al.* (2024), included maintaining microbial balance and reducing the need for pharmacological prophylaxis. In the study, similar results were provided by probiotics, which reduced endogenous stress and contributed to better nutrient absorption. The study by G. Korsa *et al.* (2024) showed the effectiveness of *Azolla* as an environmentally appropriate feed additive. Although no alternative protein sources were used in the study, probiotic intervention provided a similar improvement in protein uptake, which helped to reduce the metabolic load.

The decrease in the frequency of ARGs detection in soils after the application of biotechnology was consistent with the results of Y. Zhu *et al*. (2013), where a high density of ARGs was found in farms with frequent use of antibiotics. As part of the study, a decrease in prevalence of *tetM*, *sul1*, *blaTEM* was recorded, which indicates a reduced risk of horizontal transfer of resistance genes. R. Puchałka *et al.* (2023) demonstrated the ability of probiotic feed additives to reduce the amount of ARGs in soils fertilised with animal waste. Similarly, this study recorded a decrease in ARGs, which confirmed the effect of biological detoxification of the agroecosystem. The findings of P.P. Nagy *et al.* (2022), which emphasised the importance of selection for immune resistance, were consistent with the data obtained on reducing the pharmacological load. The use of genetically based selection reduced the frequency of therapeutic interventions without compromising weight gain. G. Wu & F.W. Bazer (2019) showed that the use of biotechnologies contributes to improved feed conversion and economic returns. The results obtained as part of the study were fully consistent with this finding, as there was an improvement in growth rates, a reduction in veterinary drug costs, and an increase in profitability.

F. Khajali & F. Rafiei (2024) have shown that controlling antinutrients in animal diets is crucial for metabolic stability. A similar effect in the study was achieved by using probiotics, which improved digestibility and had a positive effect on haematological parameters. W.M. Rauw *et al.* (2020) substantiated the need to integrate biotechnologies into the system of feeding, breeding, and management to achieve sustainability of animal husbandry. The overall results confirmed the effectiveness of a combination of probiotic, breeding, and vaccination interventions to reduce antibiotic costs and environmental burden. In the study by W. Huangfu *et al.* (2024), *in vitro* and *in vivo* models confirmed the effectiveness of fermentation technologies in reducing nitrogen losses. In the current study, a similar effect was achieved by stabilising the gut microbiota, which helped to improve protein digestion and reduce the synthesis of volatile compounds. The approach to green bioeconomics described by U. Jørgensen *et al.* (2022) provided for the integration of biotechnological innovations to improve the environmental responsibility of agricultural production. The data obtained as part of the study were consistent with this concept, since a decrease in residual antibiotics, a decrease in ammonia emissions, and an increase in the activity of soil microorganisms were recorded. As a result, the study showed that the introduction of biotechnological solutions in pig breeding provides a multi-vector effect: increases the productivity and immune stability of animals, reduces dependence on antibiotic therapy, improves the environmental characteristics of production and economic feasibility. The systematic use of probiotics, breeding, and vaccination allows not only to meet the requirements of biosafety and sustainable development, but also to form a complete circular model of agricultural production with an emphasis on preserving the health of animals, soils, and consumers.

Conclusions

The study found that all three biotechnological tools under study – probiotics, vaccines, and breeding – contributed to improving health, productivity, and environmental sustainability in industrial pig farming. The use of probiotic drugs based on *Lactobacillus spp.*, *Bacillus subtilis*, and *Enterococcus faecium* provided an increase in the average daily weight gain of pigs by 80 g (from 670 g/day in the control group to 750 g/day in the experimental group), a decrease in feed conversion from 2.9 to 2.6 kg/kg of weight gain, a decrease in morbidity from 34.1% to 15.4%, and an increase in the level of IgG immunoglobulins in serum from 12.3 to 16.1 mg/ml.

Vaccination against PRRSV, *Mycoplasma hyopneumoniae*, and *Lawsonia intracellularis* increased the safety of livestock from 92.5% to 97% and reduced the frequency of antibiotic therapy from 87 to 52 cases per 100 animals. In the vaccine group, there was also a decrease in antibiotic residues in meat to 5.6% of samples with an excess of MPR, compared with 12.5% in the control group. The highest efficiency was demonstrated by breeding: body weight gain reached 810 g/day, feed conversion decreased to 2.5, the morbidity rate decreased to 12.7%, and the safety of livestock increased to 96.1%. The content of antibiotic residues in manure decreased to 13.7 mg/kg (against 38.6 mg/kg in the control group), and ammonia emissions from manure – to 85.3 g/day/animal.

The cost-effectiveness of the analysed solutions was confirmed by ROI calculations: 287.5% for probiotics, 50% for vaccination, and 123% for breeding. The payback period for probiotics amounted to one fattening cycle, and for breeding – 3-5 years. At the ecological level, it was found that the use of manure with a reduced content of residual antibiotics contributed to an increase in the activity of soil enzymes (urease, phosphatase), stabilisation of soil pH, a decrease in the content of antibiotic resistance genes, and a reduction in the risk of eutrophication of water bodies. Limitations of the study included regional sampling, a limited follow-up period for breeding effects, and the dependence of probiotic effectiveness on feeding conditions. Further research should be directed to the evaluation of combined biotechnological solutions in the long cycle, and the study of the relationship between the microbiome of animals and soils in the context of biosafety of agricultural systems.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

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Ефективність біотехнологічних інструментів у покращенні здоров'я поголів'я свиней та екологізації виробництва

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Анотація. У контексті глобального зростання стійкості патогенів до антибіотиків та екологічних викликів у тваринництві постає потреба у впровадженні ефективних біотехнологічних рішень. Метою дослідження було встановити ефективність застосування біотехнологічних інструментів у свинарстві з огляду на їх вплив на здоров'я, продуктивність тварин і екологічну стійкість виробництва. Експеримент проводився у період з серпня по грудень 2024 року на двох свинокомплексах Кіровоградської та Вінницької областей із загальною вибіркою 240 свиней, поділених на контрольну та три дослідні групи. У дослідженні вивчався вплив пробіотиків, вакцин та генетичної селекції на приріст живої маси, захворюваність, гематологічні й імунологічні показники, частоту використання антибіотиків, екологічні параметри та економічну рентабельність. У результаті застосування пробіотиків середньодобовий приріст збільшився на 80 г, захворюваність знизилася вдвічі, а рівень імуноглобулінів зріс на 30 %. Вакцинація забезпечила підвищення збереженості поголів'я на 4,3 %, скорочення потреби в антибіотикотерапії на 40 % і зменшення залишкових антибіотиків у продукції. Генетичний добір дав найвищі результати – зростання приросту до 810 г на добу, найнижчі показники захворюваності, аміачних викидів та залишків антибіотиків. Економічний аналіз підтвердив ефективність усіх трьох підходів: рівень рентабельності інвестицій становив 287,5 % для пробіотиків, 50 % для вакцинації та 123 % для селекції. Практична ефективність результатів підтверджена прикладами впровадження аналогічних підходів у Данії, Польщі та Бразилії, де було досягнуто зменшення використання антибіотиків без шкоди для продуктивності. Дослідження також засвідчило позитивний вплив на мікробну активність ґрунтів, зменшення кислотного навантаження та ризиків евтрофікації. Результати можуть бути використані у практиці свинарських господарств для підвищення продуктивності тварин, зменшення залежності від антибіотиків і зниження екологічного навантаження на агроекосистеми

Ключові слова: пробіотики; вакцинація; генетика; тваринництво; ветеринарія