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The effect of feed additives on biochemical and immunological blood parameters in growing pigs

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Contents

Abstract

This study presents the results of biochemical and immunological indicators of clinically healthy young pigs of the Large White breed. The research was conducted against the background of expanding the basic diet with supplements having different mechanisms of action: the probiotics BioPlus 2B (based on *Bacillus licheniformis* CH 200 and *Bacillus subtilis* 201) and Bacell (*Ruminococcus albus*, *Lactobacillus* spp., *Bacillus subtilis* 8130 in composition), and a mixture of plant extracts Extract SV containing carvacrol, cinnamaldehyde, and capsaicin. Animals were randomly selected for the control group (Group 1), and for the experimental groups – according to the results of bacteriological studies of the intestinal microflora. Group 2 consisted of pigs with a reduced content of *Escherichia coli*, which consumed BioPlus 2B with the basic diet, Group 3 – pigs with a reduced content of *Lactobacillus spp.*, which consumed Bacell, Group 4 – animals with a normal ratio of *E. coli*, bifidobacteria and lactobacteria. Blood samples were taken immediately after the distribution of pigs into groups (day 0) and on days 18 and 62. When using feed additives, an increase in the concentration of total protein in pigs was observed on day 62, alongside changes in the protein profile of animals that consumed BioPlus 2B – an increase in α1-globulins by 1.33 times ($P < 0.01$), and Extract SV – β-globulins by 1.18 times ($P < 0.01$), BUN and creatinine by 10 % ($P < 0.05$), which indicated the absence of violations of the protein synthesis function of the liver. On day 18, we observed a significant increase in the concentration of IgG by 10 % in animals consuming Bacell, which may indicate a temporary immune-stimulating effect. The prevalence of *Lactobacillus* spp. and *Bifidobacterium* spp. in the intestine when consuming feed additives indicates a relationship between the structure of the microbiome and the biochemical and immunological indicators of the experimental pigs. The described approach to selecting feed additives allows us to achieve maximum efficiency and predict results in industrial pig farming, thereby optimizing the production cycle and increasing the profitability of the farm.

Keywords: growing pigs; gut microbiome; probiotics; phytobiotics; biochemical blood parameters; immunity.

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1. Introduction

The efficiency of the business process in pig farming depends on a number of factors that directly affect the animal's body. Weaning, a sharp change in diet and social environment often lead to a decrease in feed consumption and the intestinal damage (Camerlink et al., 2023; Kramarenko et al., 2024). The body becomes vulnerable to infections: a favorable environment is created for the reproduction of conditionally pathogenic and pathogenic microorganisms that are able to cause the development of diseases (Rhouma et al., 2017). Therefore, diagnostics and treatment will require significant investments. The use of antibiotics for the treatment or prevention of diseases without proper indications, or as growth stimulants, has a number of negative consequences (Albernaz-Gonçalves et al., 2022). Excessive or improper use of antibiotics eventually makes bacteria resistant to these products, and treatment becomes ineffective. Moreover, since resistant bacteria can be transmitted through the food chain or the environment, this also poses a threat to human health (Pandey et al., 2024). Given these risks, modern approaches in pig farming are aimed at minimizing the use of antibiotics by improving housing conditions, feeding, and increasing farm biosecurity (Pandey et al., 2024).

In this context, intestine health is of great importance, encompassing at least the maintenance of intestinal barrier integrity, balancing the microbiome, and the effective functioning of the immune system (Jiang et al., 2024). Immunity in pigs, as in other mammals, is realized through a complex interaction of cellular and humoral mechanisms aimed at the effective detection, neutralization, and elimination of pathogens. These factors are considered key to the survival and maintenance of health in pigs and must be taken into account (Mainardi et al., 2024).

It is known that the immune status of pigs changes with age and depends on the conditions of keeping, feeding and vaccination (Gimsa et al., 2018). Strengthening the immune system is a result of a combination of passive immunity received from the sow and the development of its own active immunity through vaccination and exposure to infections. In addition, balanced feeding with the use of appropriate feed additives is crucial.

The use of various feed additives is a contribution to the productivity of the farm. These products help to fully reveal the genetic potential of animals, minimizing the risks of diseases. But the presence of the most effective products still cannot guarantee the effect of their use, because there are certain conditions when this or that agent or biologically active additive will be maximally beneficial for the body. Given that most studies are devoted to the theoretically justified determination of biochemical and immunological indicators in pigs, we decided to investigate how these indicators change in pigs during growth when using feed additives with different mechanisms of action and considering the initial population of the intestinal microbiome.

2. Materials and methods

Animals. Research was conducted in accordance with the International Principles of the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Strasbourg, 1985) and according to the norms set forth in the current guidelines on the care and use of experimental animals, established by Order of Ukraine No 3447-IV 21.02.06 “On the protection of animals from cruelty”. Protocol of animals’ experiments was approved by the Local Ethics Review Committee of Dnipro State Agrarian and Economic University (Protocol № 17–012022, Dnipro, Ukraine).

Clinically healthy pigs of the Large White breed, aged 2–4 months, were selected for the study, taking into account their live weight, sex, and growth energy (Yanovs′ka, 2009). The animals were housed in corresponding groups in separate quarters of a pig farm in the Nikopol district of the Dnipropetrovsk region and were provided with twice-daily feeding and access to water *ad libitum*.

The basic diet of the experimental animals consisted of wheat, barley, corn groats, sunflower cake, wheat bran, dry feed yeast, table salt, chalk, premix 0.5 %, L-lysine. For research purposes, feed additives with different mechanisms of action were added to the diet. Probiotics BioPlus 2B based on *Bacillus licheniformis* CH 200 and *Bacillus subtilis* 201, Bacell with *Ruminococcus albus*, *Lactobacillus sp, Bacillus subtilis* 8130 in composition and Extract SV, which is a mixture of plant extracts containing carvacrol, cinnamaldehyde and capsaicin, were used. The addition of feed additives to the compound feed was carried out by stepwise mixing in compliance with the manufacturers' recommendations: BioPlus 2B – in the amount of 0.5 kg/t, Bacell – 2.0 kg/t, Extract SV – 0.2 kg/t.

Experimental protocol. The total duration of the study was 82 days. A two-week equalization period was used for the adaptation of the pigs, after which, according to the results of bacteriological studies of the intestinal microflora of the animals, they were divided into 4 groups of 14 animals each (day 0). Group 1 was the control group, to which piglets were randomly selected. They consumed a basic diet based on farm feed for the next 62 days. Group 2 included pigs with a reduced *Escherichia coli* content; their basic diet was expanded with probiotic BioPlus 2B. Group 3 included pigs with a reduced *L.* content, which consumed a combined probiotic Bacell. Group 4 included animals with a normal ratio of E. coli, bifidobacteria and lactobacteria, and Extract SV was added to their diet.

Further bacteriological studies in the experimental groups were carried out while using feed additives on days 18 and 62 of the accounting period. The algorithm for collecting feces and methods for conducting bacteriological studies were described previously (Yanovska & Gordiienko, 2023).

Immediately after distribution into groups and on days 18 and 62, blood was collected from the animals following all aseptic and antiseptic procedures. Blood was sampled from the ear vein in the morning before the first feeding. After clotting at room temperature, the blood was centrifuged for 15 minutes at 2000 rpm. Serum was used for further studies. The samples were frozen and stored for 2 to 6 months at –70 °C.

During the accounting period, experimental pigs were weighed monthly before the first feeding.

Biochemical analysis. Total protein, urea, and creatinine were determined in the test samples using Elitech diagnostics Seppim S.A.S test kits (France) on a semi-automatic biochemical analyzer BA-88 Mindray (China), following the instructions and application schemes provided for the analyzer. Protein was determined by the biuret method, urea – by the kinetic urease method, and creatinine – by the kinetic alkaline picrate method.

Protein fractions were detected using a PEF-3 electrophoresis device. Electrophoretic separation of blood serum samples was performed on paper in a veronal-acetate-citrate buffer (VAC-buffer) with subsequent staining with Amido Black solution. Excess dye was removed by washing using a solution of acetic acid with phenol. The paper was dried. Then, the fractions were eluted with 0.1 N sodium hydroxide. For further measurement of optical density, a spectrophotometer PV 1251 Solar (Belarus) was used at 540 nm. The results obtained were given in percentage.

Immunological analysis. The content of immunoglobulins A, M, G in blood serum was determined using test systems for the enzyme immunoassay (Granum, Ukraine). Optical density was measured using a spectrophotometer Humareader (Human, Germany) at 450 nm against air. The concentration was calculated by the formula using the inverse relationship.

Statistical analysis. Results are presented as mean value and standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by comparison of groups with Tukey's post-hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

A preliminary analysis of the microbiome structure revealed that when feed additives with probiotic action are added to the diet of pigs for growth, enterobacteria, *Proteus spp., Staphylococcus aureus, Staphylococcus saprophiticus* are displaced in favor of acid-forming microflora. Against the background of BioPlus 2B consumption, the content of *Lactobacillus* spp. increases. When adding Bacell, *Lactoba-*

cillus spp. and *Bifidobacterium* spp. prevail, whereas with the use of Extract SV, the changes are similar, only less pronounced (Yanovska & Gordiienko, 2023).

Today, the market offers a wide range of feed additives with different mechanisms of action, adapted for use in the relevant production groups of pigs (Radzikowski & Milczarek, 2021). Modern studies demonstrate multiple beneficial effects of these products, the spectrum of which depends on the type of additive (Hao et al., 2020; Boston et al., 2024; Garavito-Duarte et al., 2025). During the study, three types of additives were used: probiotics BioPlus 2B and Bacell, and phytobiotic Extract SV. In view of corresponding changes in the microbiome, biochemical and immunological indicators of pigs on growing were analyzed.

The results of biochemical studies of blood serum of experimental pigs consuming feed additives are given in Table 1.

Table 1

Parameters of protein metabolism in growing pigs supplemented with feed additives (x ± SD, n = 14)

Parameter	Day of observed period	Group 1 (Control, BD)	Group 2 (BD + BioPlus 2B)	Group 3 (BD + Bacell)	Group 4 (BD + Extract SV)
Total protein, g/L	0	68.3 ± 4.6^{a}	65.7 ± 3.4^{a}	65.4 ± 2.9^{a}	68.1 ± 2.7^{a}
	18	71.9 ± 2.9^{a}	70.3 ± 1.8^{a}	67.0 ± 3.4^{a}	71.1 ± 3.4^{a}
	62	76.0 ± 1.7^{a}	83.3 ± 0.3^{ab}	74.0 ± 0.7^{a}	82.7 ± 0.5^{ab}
Albumin, %	0	38.2 ± 0.6^{a}	39.8 ± 0.3^{a}	36.6 ± 0.7^{a}	40.1 ± 0.8^{a}
	18	39.3 ± 1.0^{a}	39.1 ± 0.5^{a}	40.2 ± 0.5^{a}	39.6 ± 0.7^{a}
	62	40.3 ± 1,1^{a}	39.7 ± 0.4^{a}	38.0 ± 0.5^{a}	41.7 ± 1.0^{ab}
α_1-globulins, %	0	12.6 ± 0.9^{a}	14.6 ± 1.8^{a}	12.1 ± 1.6^{a}	14.2 ± 1.9^{a}
	18	13.0 ± 1.0^{a}	16.5 ± 2.9^{a}	13.5 ± 0.9^{a}	15.5 ± 2.5^{a}
	62	13.3 ± 1.3^{a}	17.7 ± 0.4^{b}	13.0 ± 0.3^{a}	15.7 ± 1.6^{a}
α_2-globulins, %	0	9.2 ± 0.5^{a}	8.9 ± 1.7^{a}	9.8 ± 0.3^{a}	8.5 ± 1.5^{a}
	18	9.2 ± 0.8^{a}	8.5 ± 1.4^{a}	10.5 ± 0.8^{a}	8.7 ± 1.4^{a}
	62	9.0 ± 0,9^{a}	8.3 ± 0.7^{a}	10.0 ± 1.1^{a}	9.0 ± 0.4^{a}
β-globulins, %	0	15.4 ± 3.4^{a}	10.8 ± 2.3^{a}	14.4 ± 3.7^{a}	12.1 ± 1.7^{a}
	18	15.8 ± 3.6^{a}	11.0 ± 2.2^{a}	15.0 ± 1.6^{a}	13.5 ± 1.9^{a}
	62	16.0 ± 0.5^{ab}	11.7 ± 1.5^{a}	17.3 ± 0.4^{b}	13.3 ± 2.1^{a}
γ-globulins, %	0	24.6 ± 0.9^{a}	24.5 ± 0.5^{a}	22.0 ± 0.4^{a}	25.1 ± 0.9^{a}
	18	22.7 ± 1.8^{a}	24.9 ± 1.6^{a}	21.8 ± 1.4^{a}	22.7 ± 1.0^{a}
	62	21.3 ± 1.3^{a}	22.7 ± 0.7^{a}	21.7 ± 0.5^{a}	20.3 ± 0.7^{a}
Urea, mmol/L	0	4.1 ± 2.1^{a}	3.3 ± 0.8^{a}	4.2 ± 0.8^{a}	3.8 ± 0.4^{a}
	18	4.6 ± 0.8^{a}	4.1 ± 0.5^{a}	5.7 ± 1.9^{a}	4.8 ± 0.8^{a}
	62	5.5 ± 1.4^{a}	4.7 ± 0.2^{a}	3.6 ± 0.4^{a}	6.1 ± 0.2^{ab}
BUN, mmol/L	0	1.92 ± 0.96^{a}	1.54 ± 1.03^{a}	1.9 ± 0.34^{a}	1.78 ± 0.12^{a}
	18	2.15 ± 0.43^{a}	1.85 ± 0.68^{a}	2.66 ± 0.96^{a}	2.71 ± 0.33^{a}
	62	2.56 ± 1.27^{a}	2.19 ± 0.19^{a}	1.68 ± 0.53^{a}	2.84 ± 0.16^{ab}
Creatinine, μmol/L	0	65.6 ± 4.2^{a}	63.8 ± 2.7	66.3 ± 3.6^{a}	68.1 ± 3.5^{a}
	18	67.7 ± 1.8^{a}	67.6 ± 3.6^{a}	68.5 ± 2.8^{a}	69.9 ± 4.7^{a}
	62	71.0 ± 2.5^{a}	69.3 ± 0.7^{a}	64.0 ± 1.0^{a}	78.7 ± 1.0^{b}

Note: BD – basic diet. Significant difference among groups is indicated by different letters. Statistical analysis was done by a one-way ANOVA with Tukey's multiple comparisons post hoc test

The results presented in the work indicate the absence of significant changes in protein metabolism in animals of all experimental groups at the beginning of the study (day 0) and on day 18. When using feed additives, a moderate increase in total protein was observed in pigs at the end of the study (day 62) concurrently with changes in the protein profile: in Group 2, with an increase in the proportion of α1-globulins by 1.33 times ($P < 0.01$), and in Group 3, with the proportion of β-globulins by 1.18 times ($P < 0.01$). Given that the main blood protein is albumin, a stable albumin content may indicate the absence of impairments in the protein-synthesizing function of the liver.

In animals of Groups 2 and 3, which consumed BioPlus 2B and Bacell, the content of urea, BUN and creatinine almost did not change. In Group 4, when using the plant preparation XXX, the content of urea, BUN, and creatinine increased by 10% ($P < 0.05$) while total protein increased by 9% ($P < 0.01$). At the same time, despite a slight increase in albumin, no changes were observed in the ratio of globulin fractions. Our results are fully consistent with the study by Link R. et al. (Link & Kováč, 2006). Evaluation of the effect of BioPlus 2B® 10 based on *Bacillus subtilis* DSM 5750 and *Bacillus licheniformis* DSM 5749 (used as a drinking solution at a concentration 10 times higher than recommended) and BioPlus YC (in which the corresponding strains are used in a 1 : 1 ratio) also records the absence of significant changes, but indicates an increase in productivity (Rybarczyk et al., 2021; EFSA Panel..., 2023).

In addition, Mishra D.K. et al. also confirmed the absence of significant changes in biochemical parameters in growing finishing pigs when probiotics based on *Saccharomyces cerevisiae* NCDC 49 and *Lactobacillus acidophilus-15* were added to the diet (Mishra et al., 2016).

In commercial pig farming, the concept of ideal protein is dominant, which allows optimizing the formulation of compound feeds, effectively using valuable protein ingredients and achieving high production rates (Mulvenna et al., 2025). The diet of pigs during growth should cover the increased need for protein, which is a prerequisite for muscle mass growth, skeletal and internal organ development. The economic inefficiency of using excess protein in the diet is exacerbated by the negative environmental impact due to

intensive nitrogen excretion (Marín-García et al., 2022). Therefore, according to current data, the concentration of urea and BUN are good tools for detecting imbalance and deficiency of amino acids. Thus, it is logical to assume that the results obtained in the work indicate an optimally balanced diet for pigs not only in terms of crude protein, but also in terms of amino acid ratio.

In pig farming, the growing stage is not just an intermediate period, but an important link in the production chain, because it is at this time that key economic indicators of farm efficiency are laid. Modern genetic lines of pigs, in particular pigs of the Large White breed, demonstrate extremely high growth rates (Khalak & Gutyj, 2023). Intensive proliferation of body cells is usually accompanied by a lag in the development of the immunological system (Shao et al., 2021). In this case, a decrease in immune resistance is associated with an increase in the sensitivity of animals that grow and develop most intensively to infectious agents and stress factors. Therefore, in farms with high technological indicators, in order to reduce the risk of losing the best livestock, it is recommended to conduct immunological monitoring. The results of immunological studies of serum of pigs on growing consuming feed additives are given in Table 2.

Table 2

Alterations in immunoglobulin levels in pigs receiving feed additives (x ± SD, n = 14)

Parameter	Day of observed period	Group 1 (Control, BD)	Group 2 (BD + BioPlus 2B)	Group 3 (BD + Bacell)	Group 4 (BD + Extract SV)
Ig M, g/L	0	1.35 ± 0.41^{a}	1.26 ± 0.10^{a}	1.31 ± 0.36^{a}	1.24 ± 0.35^{a}
	18	1.33 ± 0.40^{a}	1.21 ± 0.25^{a}	1.12 ± 0.36^{a}	1.27 ± 0.61^{a}
	62	1.31 ± 0.67^{ab}	1.17 ± 0.33^{a}	1.10 ± 0.48^{a}	1.17 ± 0.80^{a}
Ig G, g/L	0	14.00 ± 0.81^{a}	13.20 ± 0.70^{a}	14.10 ± 0.33^{a}	13.90 ± 0.72^{a}
	18	14.20 ± 0.78^{a}	14.60 ± 0.34^{a}	15.70 ± 0.71^{ab}	13.40 ± 1.23^{a}
	62	14.70 ± 1.32^{a}	15.00 ± 0.27^{a}	14.70 ± 0.63^{a}	14.31 ± 1.08^{a}
Ig A, g/L	0	1.27 ± 0.34^{a}	1.28 ± 0.48^{a}	1.39 ± 0.69^{a}	1.28 ± 0.39^{a}
	18	1.51 ± 0.76^{a}	1.52 ± 0.13^{a}	1.55 ± 0.25^{a}	1.55 ± 0.40^{a}
	62	1.67 ± 0.62^{ab}	1.37 ± 0.59^{a}	1.57 ± 0.43^{a}	1.43 ± 0.47^{a}

Note: see Table 1

Despite the fact that at the fattening stage the immune system of pigs is more adapted to the influence of various stress factors, determining the level of immunoglobulins is an important diagnostic tool for assessing its condition. It is known that in response to antigenic stimulation, Ig M is the first to be synthesized (Reyneveld et al., 2020). However, during the accounting period, despite a general tendency to decrease, statistically significant changes in the level of IgM in the experimental groups were not detected. Primarily, this can be explained by the fact that at the fattening stage most pigs have already been in contact with common pathogens. Repeated contact with the same antigen causes a secondary immune response, which is characterized by the synthesis of Ig G and Ig A. Therefore, the level of Ig M during the secondary response usually remains low or increases for a short time (Hervé et al., 2022).

Ig G is the main type of antibody in the blood, which plays a crucial role in the formation of stable immunity (Reyneveld et al., 2020). During the conducted study, the concentration of Ig G corresponded to that in the control group. However, only on day 18 in Group 3 of animals consuming Bacell was there a significant increase in the content by 10 %. It is quite likely that this dietary supplement causes a temporary immune-stimulating effect.

Considering that plant extracts and essential oils exhibit immunomodulatory effects, increasing the general resistance of the young organism, the use of Extract SV did not meet our expectations. Similar results were demonstrated by Park J.-H. et al., who studied the effect of the bioflavonoid quercetin on immune parameters, growth rates, and nutrient absorption in pigs during growth, after the pigs were administered lipopolysaccharide *E. coli* (Park et al., 2020). In contrast, Fu Q. et al. recorded a significant increase in the titers of all antibodies in piglets with weakened immunity when adding resveratrol (0.33 g/kg) and *Echinacea purpurea* following classical swine fever vaccine (CSFV) and foot-and-mouth disease vaccine (FMDV) stimulation (Fu et al., 2018). Duarte M. E. et al. in their study found a significant decrease in Ig G concurrently with the use of oregano extracts, although the components in their composition are identical to Extract SV with the exception of thymol (Duarte & Kim, 2022).

Changes in IgA levels in the control and experimental groups were different: in the control group, a gradual increase in Ig A concentration was observed, while in animals consuming feed additives consumption, wave-like dynamics were observed. Given that the function of Ig A is to provide local immunity, fluctuations in the content of this immunoglobulin in pigs during growth may indicate constant or periodic contact of pigs with new pathogens through feed, water or air. Despite the general adaptability, stress factors, in particular changes in diet, can affect the structure of the intestinal microbiome and cause fluctuations in Ig A levels (León & Francino, 2022; Siemińska & Pejsak, 2022).

4. Conclusion

At the growing stage, the foundation for the health of pigs in the future is laid, aimed at minimizing the risks associated with the transition from milk to solid feed. In the context of the global problem of antibiotic resistance and the ban on the use of feed antibiotics as growth stimulants, the use of various feed additives is considered a priority approach in modern pig farming. Feed additives, entering the intestine, contribute to the colonization of beneficial microflora and create a favorable microclimate, thereby preventing the development of pathogenic microorganisms. Stable coexistence of various populations of microorganisms contributes to the digestion of feed, the development of the immune system and the general health of the piglet. Accord-

ingly, a balanced, improved diet plays a key role in normalizing the metabolic processes of animals and strengthening immunity. Additives Bacell and Extract SV exhibit similar effects, promoting the colonization of the intestine by *Lactobacillus* spp. and *Bifidobacterium* spp. Based on this, it can be argued that there is a relationship between the structure of the microbiome, biochemical and immunological indicators in the blood of experimental pigs. Therefore, if we take into account the initial population of the microbiome when selecting and introducing feed additives into the diet, their effectiveness will be optimal and the results will be predictable.

Conflict of interest

The authors of this study declare no conflict of interest.

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