

Biochemical indicators of pig blood when using a complex probiotic feed supplement

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The use of probiotics in animal feed is one of the most promising alternatives to the application of antibiotics. In order to establish the effectiveness of the use of a complex probiotic supplement (*Lactobacillus* spp., *Lactococcus* spp., Spirulinaceae, *Saccharomyces cerevisiae*, Actinomycetaceae, *Aspergillus* spp. and *Penicillium* spp.) in pig farming, two groups of pigs of the Poltava Meat breed were formed by the analog method. In the ration of the fattening pigs of the experimental group, 1.0% of the mass of compound feed was replaced with a complex probiotic supplement, which was fed during the 45 days of the experimental period at the age of 135 to 180 days. In experimental pigs, biochemical blood parameters were determined (the content of protein, lipids, cholesterol, triglycerides, activity of aspartate aminotransferase and alanine aminotransferase, the content of creatinine, glucose, calcium, phosphorus, albumins and globulins) using test kits from the company "Filisit Diagnostics" (UA). As a result of the research, it was established that with the use of a complex probiotic feed supplement, the content of total protein in the blood serum of the pigs of the experimental group was higher by 18.4%, glucose by 21.4%, aspartate aminotransferase activity by 20.4%, alanine aminotransferase by 38.0% compared to control group. In both groups, regardless of the established difference, the named indicators were within the physiological norm. The concentration of cholesterol in the blood of pigs receiving the probiotic supplement decreased by 43.2%, but the concentration of calcium (by 26.2%) and phosphorus (by 17.9%) increased. In the experimental group, the blood indicators related to the immunity of pigs improved – the content of $\alpha 1$ and $\alpha 2$ globulins decreased by 4.1% and 7.4%, respectively. Also, in the experimental group, the concentration of albumins increased by 7.5%, which probably indicates an improvement in the functional state of the liver. As a result of feeding with a complex probiotic supplement in the experimental group the albumin-globulin ratio improved, which increased by 35.0%. Thus, the introduction of a complex probiotic feed additive into the ration of pigs changed the structure of the influence of exogenous and endogenous factors on metabolism, had a positive effect on the protein profile of the blood, and caused an increase of average daily gains in experimental animals by 5.8% compared to the control group.

Keywords: pigs; feeding; probiotic feed supplement; biochemical blood parameters; total protein; lipids; albumins; globulins; factor analysis; live weight gain.

Introduction

Modern methods of raising pigs have included the use of antibiotics as standard practice for many years, which is the main reason for the rapid increase in bacterial resistance to antimicrobial drugs (Cardelle-Cobas et al., 2022; Vashchenko et al., 2022). The presence of a large number of animals kept in a limited area, often combined with sub-optimal zoohygienic conditions, contributes to the spread of zoonotic pathogens such as *Salmonella* (Correia & Gomes et al., 2021; Rodrigues da Costa et al., 2021), *Campylobacter* (Marotta et al., 2020; Rath et al., 2021; Papadopoulos et al., 2021), *Escherichia coli* (Dubreuil, 2021; Kim et al., 2022; Sukhno et al., 2022), Nematodes (Yevstafieva et al., 2016; de Araújo et al., 2020; Lindgren et al., 2020) or *Enterococcus* (Pissetti et al., 2021). This, in turn, poses a risk of environmental pollution and the emergence of resistant strains of microorganisms (Bogere et al., 2019; Luise et al., 2022), which usually reduces the average daily gains of pigs and, as a result, reduces production efficiency. All these factors force veterinarians to prescribe more and more antibiotics, increasing the likelihood of a higher

prevalence of antibiotic-resistant microorganisms in livestock (Augustyniak et al., 2018; Bogere et al., 2019). The spread of the problem of antibiotic resistance has become so global that it has necessitated the introduction of legislative restrictions at the level of Regulation (EU) 2019/6, regarding the prohibition of the prophylactic use of such drugs. The scale of this problem is comparable to the problem of resource conservation and minimization of the negative impact of livestock production on the environment (Zos-Kior et al., 2020; Brockova et al., 2021; Zos-Kior et al., 2021; Chechet et al., 2022). That is why there is a growing need to develop alternative strategies to improve animal health, biological performance and sustainable production economics (Berezovsky & Vashchenko, 2015; Vashchenko et al., 2022; Saienko et al., 2023).

One of the promising approaches to solving this problem is the use of probiotics. Probiotics can have a beneficial effect on the body of the host (for example, domestic pigs) after being taken internally by improving the balance of intestinal microflora (Rybarczyk et al., 2020; Cunningham et al., 2021; Canibe et al., 2022). The term "probiotic" refers to live microorganisms (with proven probiotic properties and origin) that, when admi-

nistered in sufficient quantities, affect the health and performance of animals (Liao & Nyachoti, 2017; Swanson et al., 2020). Currently, most of the probiotics produced use lactic acid bacteria (*Lactobacillus acidophilus*) that prevent development *Salmonella* spp, *Escherichia coli*, *Candida albicans* and stimulate immunity. Other strains of the genus *Lactobacillus* spp. synthesize vitamins D and K (*L. brevis*), produce the antibiotic lactoline (*L. plantum*) or increase lactose tolerance (*L. rhamnosus*). Other bacteria used in the production of probiotics such as *Bifidobacterium bifidum*, inhibit the development of pathogens; *Enterococcus faecium* inhibits rotaviruses (Giliński & Grzegorzczak, 2017).

It was established that addition of probiotics stimulates the immune system more effectively in piglets than in fattening pigs (Cao et al., 2020; Kang et al., 2021). This effect may be related to the maturation processes of the gastrointestinal tract and the immune system. Probiotics mainly act as immunostimulators through the pathways of cellular and humoral immunity, the production of immunoglobulins and interferon, the activation of macrophages, lymphocytes and NK (natural killer) cells, as well as the regulation of phagocytosis (Dvoroznáková et al., 2016). They make piglets less susceptible to intestinal disorders after weaning and improve their ability to digest feed components (Hu & Kim, 2020; Wang et al., 2021). In addition to the immune system, probiotics affect the intestinal microbiome, changing the composition of the microbiota. Their most common positive effect in raising pigs is a reduction in the mortality of piglets before weaning and in the early period after weaning (Sánchez et al., 2016). In addition, probiotics and prebiotics have a positive effect on animal growth performance (Dowarah et al., 2017).

Multispecies probiotics have been suggested to be more effective than single strains because additive or even synergistic effects may be achieved (Chapman et al., 2011; Pereira et al., 2022). However, it has also been pointed out that the greater diversity of probiotic strains present may reduce its effectiveness due to mutual inhibition by different species, antimicrobial compounds, or competition for nutrients or binding sites (Chapman et al., 2011). Multispecies probiotics have been found to prevent post-weaning diarrhea in pigs (Hayakawa et al., 2016; Lu et al., 2018) and improve the general condition of the intestines when using them in feed additives (Giang et al., 2012; Liu et al., 2017). Several combinations of probiotics have shown potential to mitigate the adverse effects of pathogen infection (Barba-Vidal et al., 2017; Pan et al., 2017). It was found that the addition of a probiotic complex containing *L. casei* and *E. faecalis* improved growth performance and reduced the incidence of diarrhea and mortality by improving gut health, immune status and maintaining microbial balance during weaning (Liu et al., 2017).

The beneficial effects of multispecies probiotics are associated with different mechanisms of action, including competitive exclusion of pathogenic bacteria, modulation of gut microbiota, immunomodulation, and antioxidant effects. Therefore, further research is needed to better understand symbiotic or synergistic combinations to maximize the positive benefits of probiotic combinations. In research (Rybarczyk et al., 2021) it was established that the probiotic supplement EM Bokashi leads to a significant increase in the number of lactic acid bacteria and yeast in feed, a decrease in the number of *Clostridium* spp. and Enterobacteriaceae in the mucosa and colorectal part of the intestine. The study showed that pig carcasses treated with EM Bokashi probiotic had a higher percentage of lean meat and lower fat content than carcasses of control feedlot animals. The use of EM Bokashi as a feed additive leads to an increase in the concentration of pro-inflammatory cytokines TNF- and IL-6, which, in turn, increase the protective capacity of colostrum, stimulating cellular immune mechanisms that protect the sow and newborn piglets against infection. In addition, increased concentrations of cytokines IL-4, IL-10 and TGF-, as well as immunoglobulins in colostrum and milk prove the immunoregulatory effect of EM Bokashi on Th2 cells, which leads to increased expression of regulatory T-cells and polarization of the immune response from Th1 to Th2 (Laskowska et al., 2019). Results of another study (Reszka et al., 2020) showed that EM Bokashi supplementation had a positive effect on the morphological characteristics of the pig small intestine and caused an increase in the expression of genes related to key metabolic pathways of the gastrointestinal tract.

Biochemical indicators are the most common tool for assessing the body's homeostasis. A change in the level of even one of them can serve

as evidence of metabolic disorders and the development of diseases, acting as indicators, or be the result of the influence of changing conditions, the environment or other factors (Khalak, 2021; Vashchenko & Sukhno, 2022). Factor and cluster analysis are used in the statistical processing of biochemical, as well as any other quantitative indicators. The multi-vector effect on the body of animals of multifunctional products, which includes EM Bokashi, requires further disclosure of the mechanisms of their action according to other indicators that indicate the state of their health and metabolism. Therefore, the study of relationships between individual biochemical indicators of blood under the conditions of feeding pigs with fermented feed additives made with the use of various complex probiotic preparations, which include effective microorganisms, is relevant. Taking into account the analysis of literary sources and the relevance of the topic, the goal of our article was to determine the relationships between individual biochemical indicators of pig blood and the use of the complex probiotic supplement "Bokashi EMBIOTIC".

Materials and methods

To study the effectiveness of the use of the complex probiotic supplement "Bokashi Embiotic" in pig farming, a scientific and economic experiment was conducted to study its effect on the physiological state and productivity of animals. For this, 24 head were selected and two groups of animals of the Poltava meat breed were formed, 12 head each, similar in age, sex and live weight. The average live weight of animals in the group is 65.2 ± 1.9 kg.

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 in accordance with Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes. The research program was reviewed and approved by the Bioethics Committee of the Institute of Pig Breeding and Agro-Industrial Production (protocol No. 1 dated February 5, 2018). The animals were kept in a special shed in individual pens with a sufficient area (1.2 m^2 per head) to ensure proper housing conditions, which made it possible to minimize obstacles to the animals' satisfaction of physiological and ethological needs. The floor in the pens is concrete. An underlay made of wheat and barley straw was used at the rate of 1.5 kg per animal per day. The housing system was free-range, which was due to legislative restrictions in Ukraine caused by the spread of African swine fever in the country. The ventilation of the shed was natural with the use of supply valves in the walls. During the experiment, the maximum-recorded speed of air movement in the piggery did not exceed 1.0 m/s, the concentration of ammonia did not exceed 18.2 mg/m^3 , and the concentration of carbon dioxide did not exceed 0.25%.

Feed distribution was carried out automatically using a cable-disc conveyor in metal feeders with a width of 40 cm. Manure was removed with a scraper conveyor. Experimental pigs had round-the-clock access to water. The animals were watered from automatic waterers. Control of housing conditions (microclimate parameters, availability of litter, serviceability of automatic waterers), as well as control of the health of experimental animals was carried out daily. Before putting the animals to the experiment, the shed was disinfected, and the experimental pigs were given preventive deworming. The average daily air temperature in the shed where the experimental pigs were kept was at the level of 19.92 ± 2.48 °C, the relative humidity ranged from 70–75%, which according to researchers (Lykhach et al., 2023) is the optimal level for fattening pigs.

The pigs of the first (control) group received the basic ration. In the ration of their analogs of the second (experimental) group, 1.0% of the combined feed (by weight) was replaced by the investigated feed additive "Bokashi Embiotic" (Table 1), made on the basis of wheat bran fermented by effective microorganisms (bacteria Spirulinaceae, *Lactobacillus* spp, *Lactococcus* spp, Actinomycetaceae, yeast *Saccharomyces cerevisiae*, fermenting fungi of the genera *Aspergillus* and *Penicillium*).

Chemical composition of feed additives according to zootechnical analysis: total moisture – $21.78 \pm 0.38\%$, ash – $3.12 \pm 0.12\%$, nitrogen – $2.28 \pm 0.09\%$, protein – $12.13 \pm 0.32\%$, fat – $3.05 \pm 0.11\%$, fiber – $4.16 \pm 0.20\%$, calcium – $0.257 \pm 0.008\%$, phosphorus – $0.476 \pm 0.013\%$, nitro-

gen-free extractive substances – $55.76 \pm 1.72\%$. At the beginning of the studies, at the age of 135 days and at the end of the studies at the age of 180 days, experimental pigs were weighed on an electronic scale with a

maximum weighing limit of 500 kg and a resolution of 0.2 kg (Radwag, Poland). Average daily weight gain (g) was calculated based on the results of weighing.

Table 1

Feeding ration of pigs in a study on the effectiveness of the use of the complex probiotic supplement "Bokashi Embiotic" in pig farming

Feed components	Control group	Experimental group	Nutrients	Control group	Experimental group
Barley, %	40	39	Metabolic energy, MJ/kg	12.280 ± 0.113	12.279 ± 0.126
Bokashi Embiotic	–	1	Crude protein, %	14.900 ± 0.150	14.902 ± 0.140
Wheat, %	20	20	Digestible protein, %	12.124 ± 0.112	12.125 ± 0.125
Com, %	20	20	Phosphorus (total), %	0.7225 ± 0.0069	0.7226 ± 0.0081
Sunflower cake, %	10	10	Phosphorus (digestible), %	0.4368 ± 0.0035	0.4364 ± 0.0048
Protein-vitamin-mineral supplement, %	10	10	Ca, %	0.9243 ± 0.0094	0.9244 ± 0.0086

Blood samples were taken from experimental animals for biochemical studies. Blood for biochemical analysis was taken on an empty stomach from the ear marginal vein. Biochemical indicators of blood were determined using commercial kits (Filisit Diagnostics, Ukraine) according to the methods Vlizlo et al. (2012). In blood serum, the content of total protein (g/L), the activity of aspartate aminotransferase (mccat/L), alanine aminotransferase (mccat/L), the content of creatinine (mmol/L), glucose (mmol/L), total lipids (g/L), total cholesterol (mmol/L), triglycerides (mmol/L), calcium (mmol/L), phosphorus (mmol/L) and the ratio of these two macroelements were determined. Protein fractions in blood serum were also determined, namely: the content of albumins (%), α_1 - α_2 - and γ -globulins (%); after that, the ratio of albumins to globulins (units) was determined.

Statistical processing of the obtained data was carried out using Statistica 10 (StatSoft, EU) programs. The data were previously checked for normality of their distribution using the Shapiro-Wilk W-test and the Liliefors test. The probability of difference between the experimental and control groups (P) was calculated using analysis of variance (ANOVA).

Table 2

Biochemical indicators of the protein and energy metabolism of the blood of pigs under the conditions of feeding the complex probiotic feed supplement "Bokashi Embiotic" (n = 10)

Indicators	Control	Experimental group.
Total protein, g/L	81.7 ± 4.9	96.7 ± 3.3*
Aspartate aminotransferase, μ Kat/L	0.1743 ± 0.0082	0.2154 ± 0.0078*
Alanine aminotransferase, μ Kat/L	0.125 ± 0.010	0.160 ± 0.011*
Aspartate aminotransferase / Alanine aminotransferase	1.474 ± 0.092	1.270 ± 0.068
Creatinine, μ M/L	184.4 ± 7.4	194.7 ± 5.5
Glucose, mM/L	4.21 ± 0.12	5.11 ± 0.23**

Note: the difference is significant (ANOVA): * – $P < 0.05$, ** – $P < 0.01$.

An increase in the activity of aspartate and alanine aminotransferases (AsAT; AlAT) was observed in the blood of pigs of the experimental groups, but all indicators were within the physiological norm. The level of AsAT activity in the experimental group was higher by 23.5% ($P = 0.016$). AlAT activity in animals of the experimental group was higher by 33.3% ($P = 0.014$). That indicates some activation of the processes of protein exchange in the body under the action of the investigated feed additive. A certain decrease in the variability of these indicators in the blood of animals of the experimental group was noted - the coefficient of variation of AsAT activity decreased from 10.5% in the control group to 8.74% in the experimental group. As for AlAT, its variability decreased from 18.9% to 14.3%. The De Ritis coefficient (AsAT/AlAT ratio) for both experimental groups was within the normal range, as was the concentration of creatinine in the blood serum of experimental animals of both groups. The concentration of glucose in the blood serum of pigs of the experimental group was higher by 21.4% ($P = 8.41 \cdot 10^{-3}$).

Regarding lipid metabolism indicators, namely total lipids, triglycerides and total cholesterol in the blood of experimental pigs, it is necessary to note a decrease in cholesterol concentration by 0.96 mmol/L, or 43.2% ($P = 1.13 \cdot 10^{-4}$). The concentration of total lipids and triglycerides did not fundamentally change (Table 3). A 26.2% increase in calcium concentration in the blood of pigs of the research group was revealed ($P = 8.10 \cdot 10^{-5}$). Phosphorus concentration also increased by 17.9% ($P = 9.98 \cdot 10^{-3}$). The ratio of calcium to phosphorus in all experimental pigs was within the physiological norm.

Differences were considered significant at $P < 0.05$. Construction of dendrograms was carried out using the weighted centroid method of Weighted Pair Group Method with Arithmetic Mean (WPGMC), with the Pearson metric (Amelio & Tagarelli, 2019). Factor analysis was also used, in particular its variant – the principal components analysis method (Syms, 2008). To calculate the principal components (PC), which can be interpreted as new parameters summarizing the raw data in a condensed, integrated form, the matrix of correlation coefficients $r[i,j]$ was used, which corresponds to the normalization and centering of the raw data. Correlation matrices were calculated according to Pierson (Agresti, 2012; Glantz, 2012).

Results

Research has established that the content of total protein in the blood serum of pigs of the experimental group was higher by 18.4% ($P = 0.034$). The coefficient of variation of the indicated indicator in the animals of the experimental group was lower by 5.6% compared to the control (Table 2).

The protein spectrum of blood in the animals of the experimental group underwent quite significant changes: the concentration of albumins increased by 7.48% ($P = 0.015$), which may indicate an improvement in the functional state of the liver (Table 4).

Table 3

Biochemical indicators of the exchange of lipids and minerals in the blood of pigs under the conditions of feeding the complex probiotic feed supplement "Bokashi Embiotic" (n = 10)

Indicator	Control group	Research group
Total lipids, g/L	3.30 ± 0.12	3.29 ± 0.14
Total cholesterol, mmol/L	3.19 ± 0.08	2.22 ± 0.11***
Triglycerides, mmol/L	0.564 ± 0.017	0.503 ± 0.038
Calcium, mmol/L	2.563 ± 0.053	3.235 ± 0.074***
Phosphorus, mmol/L	1.513 ± 0.066	1.780 ± 0.046**
Calcium / phosphorus	1.69	1.81

Note: the difference is significant (ANOVA): ** – $P < 0.01$, *** – $P < 0.001$.

The content of α_1 and α_2 globulins in the experimental group decreased by 4.11% ($P = 0.034$) and 7.42% ($P = 0.010$), respectively. The content of γ globulins probably did not change, only a trend towards an increase in content by 1.97% was noted. The albumin-globulin ratio in the experimental group increased by 0.21 units, or by 35.0%, and approached the optimum. In general, such changes in the protein profile of the blood testify to the positive effect of the studied feed additive on the body of pigs.

The analysis of the main components revealed that the biochemical parameters of the blood of pigs of the control group are influenced by four main factors with the influence of 38.8%, 26.8%, 24.5% and 11.9%, respectively. The total influence is 99.0%. Thus, these four factors almost completely describe the influence of external factors on the pig's body (Fig. 1).

Table 4

The protein profile of the blood of pigs under the conditions of feeding the complex probiotic feed supplement "Bokashi Embiotic" (n = 10)

Indicator	Control group	Research group
Albumins, %	37.2 ± 2.1	44.7 ± 1.3*
α1 globulins, %	11.05 ± 1.52	6.95 ± 0.53*
α2 globulins, %	18.53 ± 2.20	11.11 ± 0.42**
β globulins, %	14.16 ± 0.95	16.25 ± 1.08
γ globulins, %	19.1 ± 1.0	21.0 ± 1.8
Albumins / globulins, units	0.600 ± 0.052	0.811 ± 0.044*

Note: see Table 2.

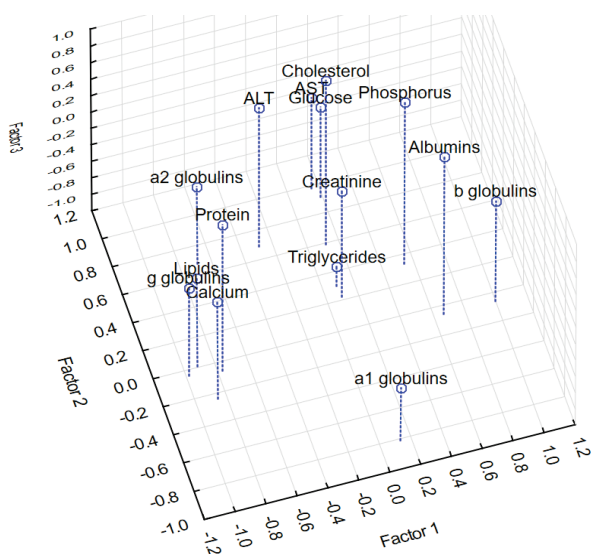


Fig. 1. Projection of control group data onto the plane of the first three factors: ALT – alanine aminotransferase; AST – aspartate aminotransferase; a1, a2 globulins – α1 and α2 globulins ; g globulins – γ-globulins

The first factor is reliably correlated with the total protein content ($r = -0.770 \pm 0.226$; $P < 0.01$), calcium ($r = -0.860 \pm 0.180$; $P < 0.01$), total lipids ($r = -0.910 \pm 0.147$; $P < 0.001$), β-globulins ($r = 0.990 \pm 0.050$; $P < 0.001$), γ-globulins ($r = -0.980 \pm 0.070$; $P < 0.001$). The second factor probably correlates with the glucose content ($r = 0.910 \pm 0.147$; $P < 0.001$), α1-globulins ($r = -0.820 \pm 0.202$; $P < 0.001$) and activity of aspartate aminotransferase ($r = 0.98 \pm 0.070$; $P < 0.001$). The third factor probably correlates with the content of phosphorus ($r = 0.770 \pm 0.226$; $P < 0.01$), triglycerides ($r = -0.950 \pm 0.110$; $P < 0.001$), total cholesterol ($r = 0.800 \pm 0.212$; $P < 0.01$), albumins ($r = 0.720 \pm 0.245$; $P < 0.01$). The fourth probably correlates only with creatinine content ($r = 0.970 \pm 0.086$; $P < 0.001$).

The analysis of the main components revealed that the biochemical parameters of the blood of the pigs of the research group are also influenced by 4 factors with the influence of 27.6%, 30.0%, 19.8% and 22.6%, respectively. The total impact is 100.0% (Fig. 2).

The first factor is significantly correlated with the content of creatinine ($r = 0.960 \pm 0.099$; $P < 0.001$), total cholesterol ($r = 0.730 \pm 0.242$; $P < 0.01$), α1-globulins ($r = 0.770 \pm 0.226$; $P < 0.01$) and γ-globulins ($r = -0.850 \pm 0.186$; $P < 0.001$). The second factor is reliably correlated with the content of calcium ($r = 0.870 \pm 0.174$; $P < 0.001$), phosphorus ($r = 0.900 \pm 0.154$; $P < 0.001$), triglycerides ($r = 0.950 \pm 0.110$; $P < 0.001$), albumins ($r = 0.720 \pm 0.245$; $P < 0.01$) and β-globulins ($r = -0.960 \pm 0.099$; $P < 0.001$). The third factor is significantly correlated with the content of total protein ($r = 0.990 \pm 0.050$; $P < 0.001$) and α1-globulins ($r = 0.890 \pm 0.161$; $P < 0.001$). The fourth is reliably to correlate with glucose content ($r = -0.980 \pm 0.070$; $P < 0.001$) and alanine aminotransferase

activity ($r = -0.900 \pm 0.154$; $P < 0.001$). As can be seen from Figure 1 and 2, the structure of the influence of the first three factors in the control and experimental groups is significantly different.

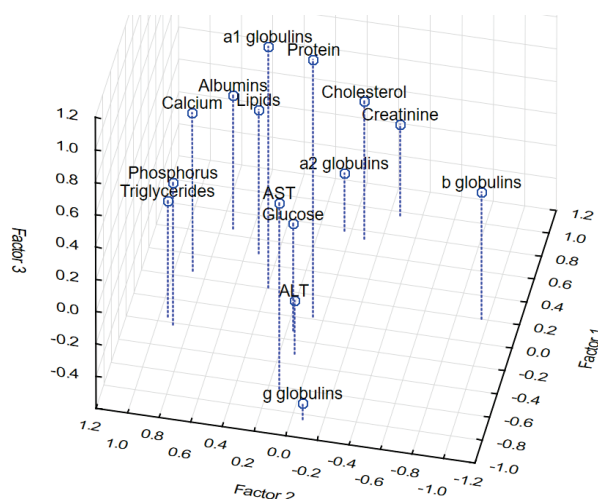


Fig. 2. Projection of the data of the research group onto the plane of the first three factors: ALT – alanine aminotransferase; AST – aspartate aminotransferase; a1, a2 globulins – α1 and α2 globulins; g globulins – γ-globulins

Cluster analysis of the biochemical parameters of the control group revealed five main clusters (Fig. 3).

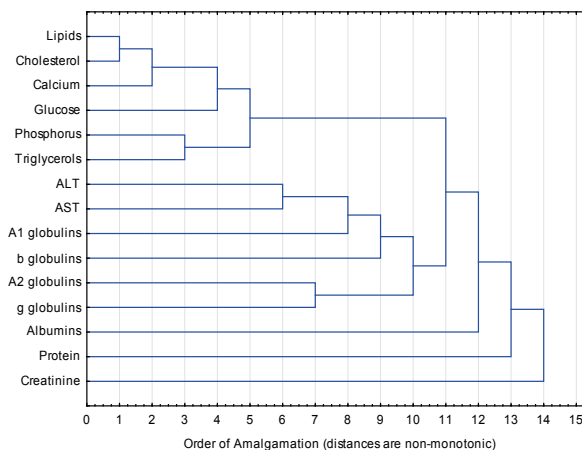


Fig. 3. Dendrogram of the relationship between biochemical parameters of the control group (the weighted centroid method of Weighted Pair Group Method with Arithmetic Mean (WPGMC), with the Pearson metric): ALT – alanine aminotransferase; AST – aspartate aminotransferase; A1, A2 globulins – α1 and α2 globulins; g globulins – γ-globulins

In the first three clusters, total protein, creatinine and albumin were identified. Cluster 4 includes aspartate aminotransferase, alanine aminotransferase, α1-, α2-, β-, and γ-globulins. In five, respectively, lipids, cholesterol, calcium, glucose, phosphorus, triglycerides. As can be seen, cluster 4 includes components of protein metabolism, and cluster 5 includes components of lipid and mineral metabolism.

The structure of the dendrogram of the experimental group differs from that of the control group. Thus, the cluster analysis of the data of biochemical indicators of the research group revealed seven main clusters (Fig. 4). In the first three clusters, as in the control, total protein, creatinine and albumin were isolated. Cluster 4 includes β- and γ-globulins, and cluster 5 includes aspartate aminotransferase, alanine aminotransferase and α2-globulins. In the sixth are glucose and α1-globulins, and in the seventh are lipids, cholesterol, calcium, phosphorus and triglycerides. As can be seen from the dendrogram, unlike the control, cluster 4 includes components of protein metabolism, and cluster 5 includes components of lipid and mineral metabolism.

The positive effect of the probiotic feed additive on the biochemical parameters of the blood contributed to an increase of the average daily gains during rearing of the animals of the experimental group by 42.08 g, or 5.76% ($P = 6.67 \cdot 10^{-4}$).

In this way, the introduction of the complex probiotic feed supplement "Bokashi Embiotic" into the ration of feeding pigs changes the structure of the influence of exogenous and endogenous factors on the metabolism of pigs and changes it in the desired direction.

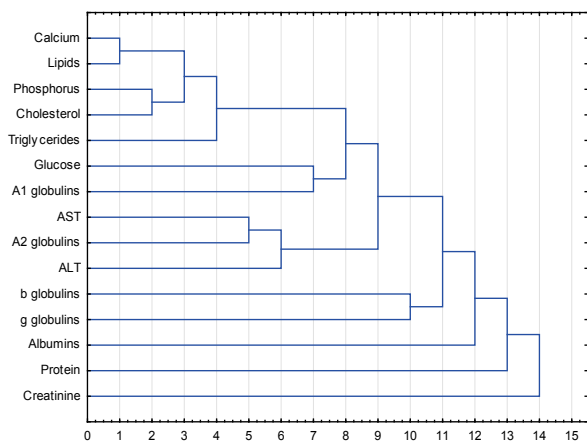


Fig. 4. Dendrogram of the relationship between biochemical parameters of the experimental group (the weighted centroid method of Weighted Pair Group Method with Arithmetic Mean (WPGMC), with the Pearson metric); ALT – alanine aminotransferase; AST – aspartate aminotransferase; A1, A2 globulins – $\alpha 1$ and $\alpha 2$ globulins; g globulins – γ -globulins

Discussion

Determining the biochemical parameters of the blood serum of experimental pigs of the Poltava meat breed revealed that feeding with a feed probiotic supplement in the ration improved the biochemical indicators of blood and enzyme activity in the experimental group. The increase in the activity of aspartate aminotransferase by 23.5% ($P = 0.016$) established in our study is consistent with the results obtained by other researchers (Czech et al., 2018) on three-breed crossbred pigs (♀ Large White × ♂ Landrace) × ♂ Duroc, where the addition of a probiotic supplement helped to increase the activity of aspartate aminotransferase ($P = 0.047$) by 21.3%. In addition, similar results were obtained for creatinine content in blood serum – in both studies, no effect of feed probiotic supplements on this indicator was found. The opposite is at work (Dong et al. 2013), a significant effect of the addition of a mixture of probiotics on the creatinine content in the blood serum of pigs was established ($P < 0.05$), which is probably explained by a much larger number of livestock involved in the mentioned studies.

However, in contrast to the results of studies on three-breed hybrids (Czech et al., 2018), where a significant difference ($P = 0.043$) was found in the content of triglycerides between the control group and animals receiving a probiotic supplement, in our research on the Poltava meat breed of pigs, no significant influence of the organized factor on this indicator was found. Only a trend was found towards a lower triglyceride content in animals that received a feed probiotic supplement.

On the contrary, in our studies, a reliable influence of the probiotic feed additive on the indicators of total protein content in blood serum ($P = 0.034$), alanine aminotransferase activity ($P = 0.014$), glucose content ($P = 8.41 \cdot 10^{-3}$) and cholesterol ($P = 1.13 \cdot 10^{-4}$) was established, while in research (Czech et al., 2018) no influence of probiotics on these indicators was found. However, when analyzing the content of different types of cholesterol (separately high-density and separately low-density), in these studies, the influence of probiotics on the content of high-density cholesterol was established ($P = 0.008$).

With regard to the content of total protein in blood serum, our research is consistent with the results of other researchers (Dong et al., 2013). In their work an increase in the concentration of this indicator was also

recorded in two-breed weaned piglets of the Great White × Landrace combination that received a mixture of probiotic bacteria *L. plantarum* and *B. subtilis*. When evaluating the content of total protein in blood serum recorded by us, it was determined that its level was within the physiological norm, which is 55–85 g/L (Novakovska, 2020), both in animals of the control and experimental groups, which indicates a satisfactory level of metabolism in both groups.

Our results are generally consistent with the research data (Ivanova & Nikolova, 2021), in which the drug Baikal EM-1 was studied. *Lactobacillus* bacteria included in Baikal EM-1 increased the activity of bile, which contributed to the assimilation of fat-soluble vitamins A, E, D and fats. The intestinal microflora was normalized, which improved the assimilation of nutrients from feed. At the age of 4 months, piglets of the experimental group that received Baikal EM-1 had a higher average live weight than the control group by 11.0% ($P < 0.05$). In our research, the addition of probiotics to the diet also improved the growth of pigs in the experimental group, which was reflected in their larger average daily gains by 5.76% ($P = 6.67 \cdot 10^{-4}$) during the period of research (from 65 kg at the time of the experiment to the weight of 100 kg at the end of the research).

In addition, the data obtained by us in the experiment are consistent with the research results Aiyegoro et al. (2017) in which researchers found no statistically significant differences in feed intake between piglets fed either one or a combination of *Lactobacillus* probiotics. But there were statistically significant differences in the average daily gain of weight.

The largest average daily gain (+28.1% compared to the control group, +29.8% compared to the group consuming the supplement with *L. reuteri* and +17.5% compared to the group consuming the supplement with *S. salivarius*; $P < 0.05$) was obtained in the group that consumed a combination of several probiotics. Greater average daily gains in the experimental group, which consumed a supplement with a complex of probiotics, determined the best payment for feed by weight gain.

Our research also benefits from work Zhao et al. (2018), when added to the diet of weaned piglets of the bacteria *Enterococcus faecium* DSM 7134 (which is included in the stock of probiotic preparations) lead to increase the average daily gain for a period of 35 days after weaning for 8.96% ($P < 0.05$).

The obtained results regarding the increase of average daily growth of the animals of the research group can be explained by the action of lactic acid bacteria included in the probiotic supplement, due to the fact that these microorganisms are capable of producing various biologically active compounds, for example, bacteriocins; organic acids, hydrogen peroxide, diacetyl and carbon dioxide (Vieco-Saiz et al., 2019). Due to specific enzymatic functions, bacteria of the *Lactobacillus* group are able to improve the absorption of nutrients, as well as stimulate the immune system of animals. Our results of research on blood parameters of animals are consistent with this statement. In both groups of pigs, all values of blood indicators were included in the reference range (Vlizlo et al., 2012).

Our results regarding a decrease in the content of $\alpha 1$ and $\alpha 2$ globulins in the experimental group by 4.11% and 7.42% ($P < 0.05$) contradict the results obtained on two-breed hybrid piglets (Dong et al., 2013), where a significant increase in this indicator was observed in the group that received probiotic supplement. The conflicting results obtained are probably explained by the different age and live weight of the experimental animals: while in our study the blood parameters of fattening pigs with a live weight of 65–100 kg were determined, in the studies Dong et al. (2013) young piglets were analyzed after weaning.

The obtained results indicate that the use of the complex probiotic supplement "Bokashi Embiotic", which is a mixture of probiotic strains and their products, in feed for pigs has a positive effect on the immune system of pigs, improves the activity of enzymes, which helps to increase the level of metabolism and increase the average daily gains.

Conclusions

The use of a complex probiotic feed additive in feeding pigs contributed to an increase in the content of total protein, glucose, calcium, and phosphorus in the blood serum of animals of the research group; the activity of aspartate and alanine aminotransferase increased, reducing cholesterol concentration. These changes indicate an improvement in me-

metabolism in the animals of the experimental group. Since such changes in hematological indicators could affect the quality composition of pig meat, it is advisable to investigate the chemical composition and physical indicators of meat quality in pigs that were fed a complex probiotic supplement.

In pigs of both groups, the De Ritis coefficient, the value of which can be used to draw a conclusion about the state of health of the animals, was within the normal range. An increase in the concentration of albumins and a simultaneous decrease in the content of $\alpha 1$ and $\alpha 2$ globulins indicate a positive effect of the studied additive on the physiological state of pigs and their immune system. In further studies, it will be desirable to investigate the effect of a complex probiotic supplement on the processes of formation of cellular immunity.

Improvement of the level of metabolism and immune status in animals that consumed the supplement contributed to increase average daily gains by 5.76%, which indicates the usefulness of complex probiotic supplements in fattening pigs.

All research was conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1985) and the Ukrainian law On the Protection of Animals Against Ill-Treatment No. 3447-IV edited on 04/08/2017.

The authors declare no conflicts of interest.

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