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Criterion for selection of feed additives with different biological effect in the feeding of growing pigs

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Abstract. To achieve the pork production profitability, it is necessary to eliminate as many unfavorable factors as possible preventing the animal productivity increase. The use of various feed additives is one of the promising directions to assess the state of health and reducing the risks of the development of pathological processes among livestock. The paper presents the results of assessing the effect of feed additives on pig growth performance depending on the initial gut microbiome of healthy store pigs of the large white breed. The composition of the gut microbial population of the experimental animals has been previously assessed, upon which the pigs have been divided into groups. Animals with reduced content of *Escherichia coli* took BioPlus 2B, with reduced content of lactobacilli – Bacell, with normal ratio of *Escherichia coli*, bifido- and lactobacilli – Extract SV. The first two drugs are probiotics, the last one is a phytobiotic with antioxidant and antimicrobial properties. Consumption of these additives together with a balanced traditional diet changed the population intestinal microflora composition in favor of *Bifidobacterium* spp. and *Lactobacillus* spp., inhibiting the growth of opportunistic pathogenic microflora having favorable effect on animal productivity. The highest increase in the total animal weight has been observed with the consumption of Extract SV ($p < 0.01$). In the future, it is planned to investigate the effect of drugs with probiotic and antioxidant properties on the productivity indicators of growing pigs under the conditions of simultaneous consumption.

Keywords: probiotics; phytobiotics; growing pigs; gut microbiome

Критерій вибору кормових добавок із різною біологічною дією в годівлі свиней на вирощуванні

Анотація. Для досягнення рентабельності виробництва свинини необхідно усунути якомога більше несприятливих чинників, що перешкоджають підвищенню продуктивності тварин. Одним з перспективних напрямків оцінювання стану здоров'я та зниження ризиків розвитку патологічних процесів серед поголів'я вважають застосування різних кормових добавок. У представленій роботі наведені результати оцінки впливу кормових добавок на продуктивність свиней залежно від первісного мікробіому кишечника здорового молодняка свиней великої білої породи. Попередньо було проведено оцінювання складу мікробної популяції кишечника дослідних тварин, на основі якого свиней розподілили по групах. Тваринам зі знизеним вмістом кишкової палички згодували BioPlus 2B, зі знизеним вмістом лактобактерій – Bacell, з нормальним співвідношенням кишкової палички, біфідо- та лактобактерій – Extract SV. Перші два препарати є пробіотиками, останній – фітобіотиком з антиоксидантними і антимікробними властивостями. Споживання цих добавок разом зі збалансованим традиційним раціоном достеменно змінювало популяційний склад мікрофлори кишечника на користь *Bifidobacterium* spp. та *Lactobacillus* spp., пригнічуючи ріст умовно патогенної мікрофлори, що сприятливо позначається на продуктивності тварин. Найвищий приріст загальної маси тварин спостерігався при застосуванні Extract SV ($p < 0,01$). У подальшому планується дослідити вплив препаратів з пробіотичними та антиоксидантними властивостями на показники продуктивності свиней на вирощуванні за умов одночасного споживання.

Ключові слова: пробіотики; фітобіотики; свині на вирощуванні; мікробіом кишечника.

Introduction

Pig breeding, as one of the ancient branches of agriculture in Ukraine, must be competitive, therefore it needs continuous improvement. Undoubtedly, the stably high pig productivity can be ensured by effective selection work, but this is not enough to achieve the pork production profitability. In addition, a few additional factors such as an increase in the feed cost, low productivity and diseases will restrain the achievement of production profitability. On the other hand, human eating behavior has long since changed: if earlier eating consisted only in satisfying hunger, now a trend has formed to satisfy nutritional needs through the aesthetics of consuming quality products to maintain health throughout life (Díaz et al., 2019; Sauerbronn et al., 2019). Accordingly, the agro-industrial complex accepts today's challenges and evolves together with the demanding consumer.

In the livestock sector, to prevent the disease development and stimulate the animal growth, farmers mainly abuse antibiotic drugs, which contributes to the antibiotic accumulation in the environment, increase in livestock morbidity, economic losses and, most importantly, devaluation of their importance for human treatment (Roca et al., 2015; Manyi-Loh et al., 2018; Rahman et al., 2022). Due to the consumption of animal products containing even a small amount of antibiotics, the microflora gradually acquires resistance to their action, which causes the development of long-term, incurable infectious diseases and quite often death (Manyi-Loh et al., 2018).

Therefore, this problem requires an emergent solution. Against the background of optimizing the use of antibiotics by both animals and humans, it is suggested to search for alternative means without negative effect on the body. Antimicrobial lipids, contained in breast milk, skin and mucous membrane of mammals, hold a special place among such drugs and are considered key components of innate immunity. These are mainly short- and medium-chain fatty acids and monoacylglycerols, especially glycerol monolaurate (Fouhse et al., 2016; Fortuoso et al., 2019; Zhou et al., 2019). But some short-chain fatty acids, for example, acetic, propionic, butyric acids, can also cause a complex effect on the intestinal epithelium, stimulating the growth of villi, crypt mitosis, and a pronounced anti-inflammatory effect (Vasquez et al., 2022).

The peculiarity of the effect of antimicrobial lipids lies in the ability to stimulate the formation of another group of antimicrobial substances – low-molecular peptides. They are found not only in mammals, but also in amphibians, insects, and microorganisms, and are necessary for host defense (Huan et al., 2020). In addition, the properties of antimicrobial peptide polymers created using genetic engineering methods are being studied (Lam et al., 2016). However, the expensive construction of any peptide variants and the search for mechanisms to achieve proteolytic stability so far prevent the mass production of these substances for practical use (Jia et al., 2019; Torres et al., 2019; Huan et al., 2020).

It is equally important to improve the quality of compost products by preventing the spread of antibiotic resistance genes in the environment. In this regard, methods of bioelectrochemical processing, nanofiltration and reverse osmosis are used (Lan et al., 2018; Ma et al., 2019). But the high cost of these methods has limited their use in practice (Feng et al., 2021). Encouraging results have been obtained when adding humic acids to compost, which not only improved the compost, but also accelerated the removal of antibiotic resistance genes and, accordingly, reduced their circulation in the natural environment (Abdellah et al., 2023).

All these approaches wedge into the production cycle the “gut health” concept, consisting at least in efficient digestion, nutrient absorption, and well-balanced animal microbiome (Kogut & Arsenault, 2016; Celi et al., 2017). The symbiotic interaction of indigenous microflora contributes to the creation on the large intestine mucosa of a biofilm from microorganisms, their metabolites

such as exopolysaccharides, mucus, and immunoglobulin A. The biofilm is involved in maintaining natural homeostasis and provides resistance to certain factors, such as the host's immune system or the effect of antibiotics (Nesse et al., 2023). In studies using salmonella, staphylococcal and clostridium bacteriophages added to the diet of weaned piglets, improvement in the gut microbiome composition has been clearly demonstrated (Kim et al., 2014; Zeng et al., 2021). These drugs have also been shown to be effective growth promoters for growing pigs whose diet does not contain antibiotics (Gebru et al., 2010).

In Ukraine, in the livestock field, the use of feed additives with probiotic effect, which have partially substituted feed antibiotics, is considered a promising direction for assessing of health and reducing the risks of the development of pathological processes among livestock. Probiotics are living microorganisms and products of their vital functions positively effecting on the host's metabolic processes (Markowiak & Śliżewska, 2018). To correct the microbiome, the use of prebiotics, which are undigestible food components capable of enhancing the growth and activity of normal intestinal microflora, is considered reasonable (Markowiak & Śliżewska, 2018; Duan et al., 2019). Due to the combined introduction of prebiotics and probiotics (synbiotics) into the balanced diet of animals, it became possible to increase their productivity, fertility, and health (Polishchuk & Bulavkina, 2010; Sidashova et al., 2014; Podobied, 2018). However, the experts' opinions regarding the use of these additives differ significantly: on the one hand, they claim that probiotics are non-toxic, have no contraindications and do not cause side effects. On the other hand, they assume that the side effects of probiotics depend on the immune status and physiological state of the host's body and can be specific for a certain strain of microorganisms and therefore require strict adherence to the application protocol (Speiser, et al., 2015; Oliveira et al., 2017; Alayande et al., 2020; Azizi et al., 2022). Unfortunately, modern recommendations do not have clearly defined requirements for the use of feed additives. Therefore, since the commercial breeding of pigs depends on the health and survival of youngsters, the purpose of this study was to find out additional criteria for selection of feed additives in the practice of feeding growing pigs.

Materials and methods

The study was conducted during 82 days of spring-summer period on a swine farm “Agro-Elita” LLC of the Nikopol district of the Dnipropetrovsk region. According to the established procedure, healthy growing pigs of the large white breed of 2-4 months of age have been selected, considering live weight, sex, growth power (Kozyr' & Svezhentsov, 2002; Yanovska, 2009). The adaptation period lasted two weeks. During the transition period, all animals have been divided into groups according to the results of bacteriological studies of intestinal microflora. Further bacteriological studies have been carried out against the background of the use of feed additives on the 18th and 62nd day of the observed period.

The fecal samples have been taken from the rectum during defecation. The samples shipped to the laboratory during 2 hours maximum. For bacteriological study, feces were weighed, suspended in sterile saline solution (1:9) and serial dilutions were prepared. Samples have been sown on selective differential culture media: Endo medium for enterobacteria isolation, Olkenytskyi medium – for *Escherichia coli* (*E. coli*) identification with reduced enzymatic activity, Ploskiriev medium – salmonella and shigella, Blickfeldt medium – lactobacilli, thioglycollate medium – bifidobacteria, egg-yolk salt medium – staphylococci, 5% blood agar – streptococci and hemolytic microorganisms, Sabouraud's medium – yeast-like fungi. Cultures have been incubated in a thermostat for 24 hours at 37°C for bacteria and 24 hours at 25°C with subsequent incubation for 72 hours at room temperature for fungal flora. Further identification

of microorganisms has been carried out according to biochemical, morphological and tinctorial properties. After incubation, the number of colonies was counted, considering sample dilution, and the arithmetic mean was calculated. The results were given in the form of a decimal logarithm of the number of colony-forming units in 1 g of feces.

The 1st control group included randomly selected growing pigs that followed a balanced diet based on farm feed (n = 14) for 62 days. The animals of all other groups received appropriate feed additives to the basic diet: probiotic BioPlus 2B with *Bacillus licheniformis* and *Bacillus subtilis* in the composition, combined probiotic Bacell based on *Ruminococcus albus*, *Lactobacillus spp.*, *Bacillus subtilis* and Extract SV being a mixture of plant extracts containing carvacrol, cinnamaldehyde and capsaicin. The 2nd group included pigs with reduced content of *E. coli* (n = 14), the

3rd – animals with reduced content of *Lactobacillus spp.* (n = 14), and the 4th – with normal ratio of *E. coli*, bifido- and lactobacilli (n = 14). The drugs were added to compound feed according to the manufacturers' recommendations by stepwise mixing: BioPlus 2B was added in the quantity of 0.5 kg/t, Bacell – 2.0 kg/t, Extract SV – 0.2 kg/t.

The experimental pigs were weighed before the first feeding once a month.

The animals were kept in groups in separate pig pens with twice-daily feeding and access to water *ad libitum*.

Results are presented as mean value and standard deviation. Statistical analysis has been performed using one-way analysis of variance ANOVA followed by comparison of groups through Tukey's test. Value of p < 0.05 has been considered statistically significant.

Table – Gut microbiome of piglets on the background of the feed additives consumption (lg CFU/g, x ± SD)

No	Microorganisms	Day of observed period	1 st group (control, BD)	2 nd group (BD + BioPlus 2B)	3 rd group (BD + Bacell)	4 th group (BD + Extract SV)
1	Low activity form of the <i>E. coli</i>	0	8.30 ± 3.88 ^{ab}	6.00 ± 2.91 ^a	8.48 ± 3.63 ^{ab}	8.25 ± 4.58 ^{ab}
		18	8.17 ± 4.02 ^a	8.38 ± 3.76 ^a	8.23 ± 3.98 ^a	8.18 ± 3.02 ^a
		62	8.08 ± 3.45 ^a	8.61 ± 2.64 ^a	8.38 ± 4.15 ^a	8.23 ± 4.75 ^a
	<i>E. coli</i> Lac–	0	7.30 ± 2.08 ^a	6.69 ± 3.01 ^a	7.48 ± 3.38 ^a	6.60 ± 4.14 ^a
		18	5.53 ± 2.69	0	0	0
		62	6.00 ± 4.11	0	0	0
2	<i>Klebsiella spp.</i> , <i>Enterobacter spp.</i> , <i>Citrobacter spp.</i> etc.	0	3.47 ± 1.39 ^a	3.30 ± 1.45 ^a	3.39 ± 1.44 ^a	3.47 ± 1.56 ^a
		18	4.38 ± 2.75	0	0	0
		62	3.94 ± 1.58	0	0	0
3	<i>Proteus spp.</i>	0	4.00 ± 2.17 ^a	3.18 ± 1.74 ^a	3.40 ± 1.52 ^a	3.78 ± 1.37 ^a
		18	4.98 ± 2.66	0	0	0
		62	5.16 ± 2.02	0	0	0
4	<i>Bifidobacterium spp.</i>	0	7.17 ± 3.98 ^a	6.48 ± 3.44 ^a	9.01 ± 3.32 ^b	8.31 ± 4.18 ^a
		18	7.00 ± 2.55	0	0	0
		62	7.30 ± 3.66 ^a	7.48 ± 3.02 ^a	9.89 ± 4.17 ^b	8.65 ± 3.66 ^{ab}
5	<i>Lactobacillus spp.</i>	0	5.30 ± 2.57 ^a	6.90 ± 3.78 ^{ab}	6.69 ± 3.29 ^a	6.30 ± 4.01 ^a
		18	5.60 ± 2.20 ^a	7.53 ± 3.59 ^b	8.57 ± 3.89 ^c	6.85 ± 3.54 ^{ab}
		62	5.41 ± 2.55 ^a	7.60 ± 3.11 ^b	7.85 ± 3.78 ^{ab}	6.64 ± 3.32 ^a
6	<i>Streptococcus lactis</i>	0	5.30 ± 2.78 ^a	7.96 ± 2.51 ^b	4.00 ± 2.21 ^a	6.76 ± 3.78 ^{ab}
		18	6.86 ± 3.05 ^a	7.76 ± 3.13 ^{ab}	6.85 ± 4.88 ^a	6.08 ± 3.07 ^a
		62	6.04 ± 3.43 ^a	7.86 ± 3.21 ^b	6.92 ± 3.75 ^a	6.85 ± 3.88 ^a
7	<i>Staphylococcus saprophiticus</i> (<i>S. saprophiticus</i>), <i>S. epidermidis</i>	0	4.00 ± 2.11 ^a	3.95 ± 1.96 ^a	3.93 ± 1.76 ^a	4.71 ± 2.44 ^a
		18	3.78 ± 1.55 ^a	3.30 ± 1.24 ^a	3.60 ± 1.96 ^a	3.42 ± 1.44 ^a
		62	3.70 ± 2.02 ^a	0	2.48 ± 1.55 ^a	0
8	<i>S. aureus</i>	0	2.86 ± 1.37 ^a	2.82 ± 1.24 ^a	3.84 ± 1.89 ^a	4.08 ± 2.80 ^a
		18	0	0	0	0
		62	0	0	0	0
9	Yeast-like fungi	0	2.95 ± 1.88 ^a	3.69 ± 2.15 ^a	3.07 ± 1.17 ^a	3.88 ± 1.54 ^a
		18	2.70 ± 1.22 ^a	3.48 ± 1.74 ^a	3.18 ± 1.69 ^a	3.49 ± 1.68 ^a
		62	2.90 ± 1.59 ^a	2.95 ± 1.78 ^a	2.85 ± 1.78 ^a	3.04 ± 1.34 ^a

Note. BD – basic diet. The data highlighted in black bold refers to the detection of *S. epidermidis*, while the *S. saprophiticus* growth has not been observed, in red – corresponds to the number of sown molds. 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.

Results

According to our data, it has been established that the microbiome of the control group of growing pigs is represented by a wide spectrum of normal and opportunistic pathogenic microflora (Table).

Among the total number of microorganisms in piglets, the major part is accounted for representatives of obligate microflora – *Bifidobacterium spp.* and *Lactobacillus spp.* At the same time, almost the same amount of opportunistic pathogenic microflora is determined: weakly fermenting and lactose-negative forms of *E. coli* prevail in animals of this group. Under the physiologically normal state, in addition to bacteria, fungal flora has been also detected. Pathogenic microorganisms *Clostridium spp.*, hemolytic *E. coli*, etc. have not been detected, except for a small quantity of *Staphylococcus aureus*.

The feed additives in the animal diet changed microbial population. In all groups of experimental animals, only *E. coli* with reduced fermentation ability has been detected and no *E. coli* with pronounced enzymatic activity has been revealed. In general, against the background of drug consumption, there is a change in the ratio of obligate and opportunistic pathogen microorganisms due to the displacement of enterobacteria, *Proteus spp.*, *S. aureus*, *S. saprophiticus*. The consumption of BioPlus 2B probiotic led to a change in the microbial population in favor of acid-forming microflora – *Lactobacillus spp.* The combination drug Bacell causes in pigs of the third group a significant growth of lactobacilli and bifidobacteria. It is worth noting that on the 18th day of the experiment, an increase in the number of *Lactobacillus spp.* has been noted in all groups under study, except for the control, against the background of the temporary disappearance of *Bifidobacterium spp.*, although at the end of the study the *Bifidobacterium spp.* growth has been recommenced. In respect with consumption of phytobiotic by the 4th group of pigs, the intestinal microflora spectrum remained the same, only the changes have been less pronounced in compare to the animals of 2nd and 3rd groups.

During the experiment, the health of the pigs was satisfactory, no symptoms of any disease were noted. The absence of signs of dyspeptic disorders such as abdominal distension testified to the normal state of the motor-evacuation function of the digestive tract in all groups of animals.

The consumption of feed additives leads to an increase in the productivity of growing pigs. Characteristically, against the background of insignificant average daily changes (Fig. 1A), there is a significant overall total growth of the live weight of animals (Fig. 1B). The highest productivity indicators have been recorded in piglets of the 4th group against the background of moderate growth in all other groups.

Discussion

It is known that the species composition and quantitative ratio of microorganisms mostly varies depending on age, sex, diet, functional state of the digestive tract, diseases, and influence of other exo- and endogenous factors (Bian et al., 2016; Stokes, 2017). The environment change, adaptation to new conditions and feeding habits can be accompanied by stress provoking the violation of physiological processes and immunity. Under such circumstances, diarrhea can occur, which is the main cause of losses in pig breeding (Frese et al., 2015; Rhouma et al., 2017). Since normal intestinal microflora is involved in many physiological processes, such as motor control, membrane digestion processes, formation of resistance to pathogenic microorganisms, maintenance of immunity by induction of T cells, synthesis of non-specific protective factors, etc., it is important to find fast and effective ways to prevent dysbiosis. To solve this problem in pig breeding, they try to use the safest possible means. Recently, a lot of attention has been paid to feed additives with different properties. The criteria for selection of such additives are mainly formed considering the pharmacokinetics and pharmacodynamics of probiotic drugs in the animal body, composition, and introduction method (Sidashova et al., 2014; Hai, 2015; Alayande et al., 2020). However, the issue of the original background of natural intestinal microflora of animals has hardly been studied. The main hypothesis of the conducted research was the assumption that the basic microbial composition of the intestine is important for animal productivity increase. In most studies carried on in this direction all pigs are randomly divided into groups and only then the specific drug is administered (Wang et al., 2020; Sun et al., 2023). To favored to certain feed additives, we suggest considering the initial intestinal microbial population.

The results obtained during the work point to the fact that when using feed additives BioPlus 2B, Bacell and Extract SV in

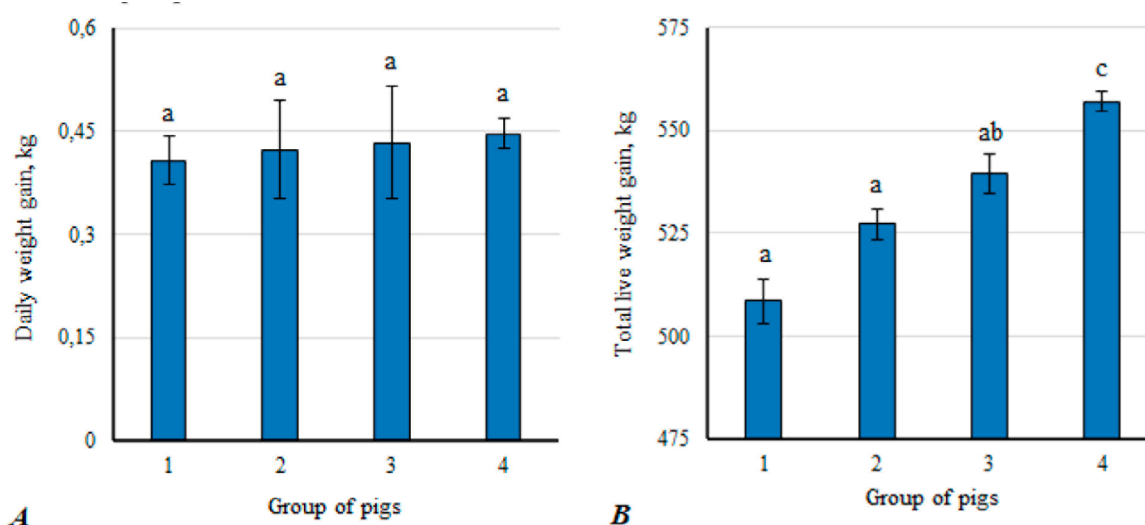


Fig. 1. Growing pigs' productivity of through the use of feed additives: (A) daily and (B) total weight growth. 1st group – the control one, the animals of which consumed the basic diet (BD), 2nd group – BD + BioPlus 2B, 3rd group – BD + Bacell, 4th group – BD + Extract SV.

the feeding of healthy pigs, there are qualitative changes in the gut microbiome in favor of obligate microflora. When using BioPlus 2B, this effect is probably provided by the peculiarities of *B. subtilis* and *B. licheniformis*. These spore-forming bacilli are resistant to changing environmental conditions and can synthesize several compounds with immunomodulatory properties and other important substances such as dipicolinic and lactic acids, bacteriocins, vitamins, oligo- and polysaccharides, short-chain fatty acids, etc., which together provide antagonistic properties regarding pathogenic and opportunistic pathogenic flora (Balciunas et al., 2015; Mikkilä et al., 2019). In addition, *Bacillus spp.* after microbiome stabilization is capable of self-elimination without disturbing the composition of intestinal microbial population. That is, these bacilli create favorable conditions for the growth of *Escherichia coli* and Gram-positive flora, in particular *Lactobacillus spp.* Similar results have been obtained during the study of the effect of drugs based on *B. megaterium* and *B. coagulans* on the pig body (Bakun et al., 2021). However, it has been established that not all Bacillus-based probiotics are equally effective. He et al. (2020) argue that different strains within the same species can have different effects on the microbiome, health, and productivity of pigs. It has been clearly shown that *B. pumilus* DSM 32540 is more effective than *B. pumilus* DSM 32539. Even if the latter has a general healthful effect on the body, it has a limited effect on pig productivity (He et al., 2020). That is, when comparing the results of various studies, it is at least worth considering the individual properties of the strains of microorganisms used. The meta-analysis conducted to determine the effect of Bacillus-based probiotics on animal body showed that the concentration of probiotics, the general health status and the age of pigs are also important in assessing animal productivity (Mun et al., 2021).

Since probiotics may contain the same microorganisms, their effects may be somewhat similar. Bacell preparation, except for *Lactobacillus spp.* and *Ruminococcus albus*, also contains *B. subtilis*, which creates conditions for the rapid growth of other microorganisms. The results of our research show an increase in the growth of lactobacilli in pigs of the second group, having a positive effect on the total daily weight growth of growing pigs. The obtained data are consistent with the results of Duan et al. (Duan et al., 2023). Instead, Huang et al. demonstrated that the consumption of probiotics with lactobacilli in the composition effectively affects daily weight growth only during the first two weeks of life (Huang et al., 2004).

The gut microbiome correction is not always achieved by consuming probiotics only. Adding certain substrates to the diet of animals can also stimulate the development of commensal microflora. Synbiotics are a successful combination of drugs with probiotic and prebiotic effect (Duarte et al., 2020; Munezero et al., 2023). They not only positively affect the animal productivity, but also influence product quality (Saracila et al., 2021). In addition, there are drugs that can indirectly affect the gut microbiome. For example, phytobiotics based on plant extracts. They have no nutritional value, but have antioxidant and antimicrobial properties, have a favorable effect on the body in general and of gut functions particularly, inhibiting pathogenic microflora (Wang et al., 2014; Rueda et al., 2021). We have demonstrated that the Extract SV phytobiotic has not been inferior to probiotic drugs as for final effect, and in terms of productivity it turned out to be the most effective feed additive: the phytobiotic consumption has been accompanied by the highest increase in the total animal weight. Similar data have been obtained while comparing the effect of phytobiotic based on carvacrol, thymol, γ -terpinene, and *p*-cymene. The consumption of these phytobiotics stimulate oxidative stress reduction and the immune status improvement of experimental animals. This leads to pig productivity increase. However, this effect is eliminated by the simultaneous introduction of phytobiotic with antibiotics used as pig growth promoters (Duarte & Kim, 2022).

Conclusions

Since the consumption of various feed additives together with basic diet changes the population composition of the intestinal microflora of clinically healthy growing pigs, to increase the productivity, these drugs should be administered differentially, taking into account the composition of the initial gut microbiome of animals, the composition and biological effect of the drug. The highest total weight growth of animals has been observed when feeding the antioxidant Extract SV drug to pigs with normal ratio of *Escherichia coli*, bifido- and lactobacilli. In the future, it is planned to investigate the effect of drugs with probiotic and antioxidant properties on the productivity indicators of growing pigs under the conditions of simultaneous consumption.

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