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Original article

EFFECTS OF METABIOTIC *BACILLUS SUBTILIS* FOR MICROBIOCENOSIS FORMATION IN EARLY-WEANED PIGLETS

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Summary

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This paper discusses the results of research related to cultivation of the probiotic microorganism *B. subtilis* on a grain-based nutrient medium of naked oat (*Avena nuda)* and studies the possibility of using metabolites as a liquid dietary supplement to correct microbiocenosis gastrointestinal tract disorders in early-weaned piglets. The weight fraction of proteinogenic amino acids in the experimental probiotic suspensions was determined by means of capillary electrophoresis. The metabolic activity of *Bacillus subtilis* DSM-32424 strain was studied on both sprouted as well as non-sprouted naked oat (*Avena nuda*) 'Nemchinovsky' variety grain-based nutrient medium. It was proved that the maximum microbial count reached 4.9×10^7 CFU/cm^3 on the 6th day of cultivation on the medium based on sprouted naked oat. The use of dietary supplement based on *Bacillus subtilis* DSM-32424 strain in amount of at least 1×10^9 CFU/pig had a positive impact in terms of the formation of microbiocenosis in animals at the time of transferring piglets to plant diet.

Key words: dietary supplement, *Enterobacteriaceae*, metabiotics, microbiocenosis, nutrient medium, piglets, probiotics

INTRODUCTION

Probiotics are living microorganisms and substances of microbial origin able to provide beneficial effects on physiological functions and biochemical reactions of the body given the natural process of their administration (Shenderov, 2013; Pajarillo *et al.*, 2015; Ardatskaya *et al.*, 2017; Plotnikova, 2018).

Published research (Patel & DuPont, 2015; Bai *et al.*, 2017; Rusaleyev *et al.*, 2019) suggest that the interaction between microorganisms in nature is manifested, among other ways, through their antagonism. Antagonism consists in the fact that bacteria from one species, when developing together with another species, can inhibit its vital activities. This bacterial property is of practical use in veterinary medicine owing to the use of bacteria as probiotics. It is proved that the widespread administration of various probiotic products from living lactobacilli and bifi-

dobacteria in veterinary and medical practice over the past decades has resulted in decrease of their therapeutic action, prompting scientists to seek novel and more effective microorganisms with probiotic properties (Rusaleyev *et al.*, 2019).

It is noteworthy that alongside the search for more effective probiotics, a scientific justification was developed in support of the administration of metabiotics as an extension of the probiotic concept. Thus, the elaboration of the probiotic concept through the study and use of metabiotics of probiotic microorganisms in order to develop novel dietary supplements and methods for their use in animal husbandry was implemented (Popov *et al.*, 2020a,b).

Metabiotics are a group of medications that contain active metabolites (products of vital functions) of the probiotic cultures, among which lysozyme, bacteriocins, catalases, enzymes, organic and amino acids, polypeptides and other compounds (Neschilyaev *et al.*, 2016; Sánchez *et al.*, 2016; Fisinin *et al.*, 2017). In addition to the bioactive action on the host, they greatly amplify antagonistic action of the producing probiotic strain, thus creating favourable conditions for its vital activity and integration into the microbiome community of gastrointestinal tract (Ushakov *et al.*, 2015; Uyeno *et al.*, 2015; Magomedaliyev *et al.*, 2020).

The clinical performance of dietary supplements based on probiotic products in the formation of microbiocenosis largely depends on biological and chemical composition of gastrointestinal environment, and manifests itself only during the period of administration owing to unfavourable conditions for assimilation. Thus, it is necessary to look for ways to prolong the effect of probiotics' usage. Still, is it compulsory to highlight the essential role of 'normal microflora' for the formation of microbiocenosis of gastrointestinal tract in animals (Sánchez *et al.*, 2016). Microflora is directly involved in digestive processes, and produces enzymes necessary for metabolism of proteins, carbohydrates, lipids and nucleic acids. When autoflora is dying out, the body system digests and absorbs it as a source of protein (Pritychenko *et al.*, 2012; Bekhtereva *et al.*, 2014; Uyeno *et al.*, 2015; Roselli *et al.*, 2017).

Consequently, the production of metabolite-type dietary supplements enriched with cell constituents of bacterial producers is a relevant topic in the field of biotechnology and animal nutrition.

The purpose of this research was to study the influence of *B. subtilis* metabolites on the formation of microbiocenosis of the gastrointestinal tract in early weaned piglets.

MATERIALS AND METHODS

The research studies took place in the Laboratory of Agrobiotechnology of Federal State Budgetary Scientific Institution Kursk Federal Agrarian Scientific Center (FSBSI Kursk FASC) in Kursk, the Russian Federation. The grains of naked oat (*Avena nuda*) in its 'Nemchinovsky' variety served as nutrient medium in order to produce metabolites in the culture of *Bacillus subtilis* DSM-32424 strain; and were provided by the Department of selection and seed farming at FSBSI Kursk FASC. All experiments were conducted in compliance with ethical standards, in accordance with Directive 2010/63/EU of the European Parliament and Council of the European union as of September 22, 2010 – On the protection of animals used for scientific purposes).

The nutrient medium used to produce metabolites of the *Bacillus subtilis* DSM-32424 culture contained sprouted and non-sprouted grains of naked oat (*Avena nuda*), 'Nemchinovsky' variety at the ratio of 100 g of crushed raw material per 3 L of water. The substance was gradually heated up at temperature from 25 to 90 °C for 6 hours. Then it was left to cool down naturally. Under control through a pH-meter, pH was adjusted to 7.5 with a 20% aqueous solution of NaOH. *B. subtilis* inoculation was done with culture previously standardised in laboratory conditions up to 1×10^6 CFU/cm^3 at a ratio of 4 mL per liter of the prepared nutrient medium.

Cultivation lasted 14 days in a KBCG 100/250 thermostat (Premed, Poland) at 37±1 °C. The number of *B. subtilis* CFU in the culture fluid was monitored on a daily basis on a Levenhuk 740T microscope (Guangzhou Jinghua Optics & Electronics Co., Ltd., China), a Levenhuk M1400 PLUS digital camera (Guangzhou Jinghua Optics & Electronics Co., Ltd., China) and a pH-meter Kelilong pH-013 (Kelilong Instruments, China).

The weight fraction of proteinogenic amino acids in experimental probiotic suspensions (EPS) was determined by means of capillary electrophoresis according the procedure stipulated by GOST R 55569-2013. The calculation of weight fraction of crude protein in g/L was carried out in accordance with GOST 32044.1-2012.

The scientific and economic analysis of experimental probiotic suspension effect on the formation of microbiocenosis of piglets' gastrointestinal tract took place on the premises of the Pig Complex 'Nadezhda' in Kursk region during rearing of weaning piglets up to the age of 75 days. Three groups of piglets aged 10 days were formed, with 15 animals in each group. Group 1 received EPS based on sprouted avena; Group 2 received EPS based on whole-grain avena; Group 3 was control. The experimental dietary supplement sample was evaporated in amount of at least 1×10^9 CFU/mL *Bacillus subtilis*. The analysis of microflora composition in faeces took place on the 10th, 25th (prenursery piglets) and 40th days (early-weaned piglets) of life by means of a quantitative cluster analysis of the large intestine content (Kondrakhina *et al.*, 2004).

Sampling was done directly from the rectum onto sterile dishes. The initial dilution was obtained in the following way: 1 g of the weighed faecal matter was well homogenised with 9 mL sterile buffer solution. A number of subsequent dilutions were produced with a buffer solution from 10^{-3} to 10^{-10} of the initial dilution, and then inoculated on the corresponding nutrient media. Incubation of all grains was performed at 37–38 °C. The assay content of bifidobacteria was determined using the dilutions of 10^{-5}–10^{-10} diluted in Blaurock semi-liquid modified hepatic medium. The *Lactobacillus* count was obtained from dilutions of 10^{-5}–10^{-9} in thin fat-free milk. Enterococci were marked out on the Alkaline polymyxin medium for the isolation of enterococci from dilutions of 10^{-3}–10^{-7}. Staphylococci were detected by inoculation on yolk salt agar (YSA) from dilutions of 10^{-4}–10^{-6}. Yeast-like fungi of the genus *Candida* were revealed on the Saburo medium containing levomycetin from dilutions of 10^{-3}–10^{-6}. *Proteus* genus members were detected on slant agar by Shukevich's method in dilutions of 10^{-1} to 10^{-4} (Kislenko, 2005). Spore-forming anaerobes (clostridia) were identified by inoculation of the suspensions of dilutions

10^{-2}, 10^{-5} and 10^{-6} into a melted column of Wilson-Blair agar medium. The total aerobic plate count and their haemolytic properties were analysed after inoculation of dilutions of 10^{-5}–10^{-7} on 5% blood agar. The potentially pathogenic enterobacteriaceae were spotted by inoculation of a drop of suspension (0.05 mL) from dilutions of 10^{3}–10^{8} into four sections of a plate containing Levine's medium. The total count of *Escherichia* was determined from dilutions of 10^{-5}–10^{-8} on the Levine's medium. The 10^{-3} dilution of was used for inoculation on Ploskirev's medium, and 10^{-5} dilution: on Endo agar. The specific media involved in the research studies were purchased from the Federal Budgetary Institution of Science State Scientific Center for Applied Microbiology and Biotechnology (FBIS SSC AMB) in the regional settlement of Obolensk in Serpukhov, Moscow Region, Russia. The content of all types of microorganisms in 1 g of faeces was evaluated through the amount of colonies grown on the corresponding medium and taking into account the inoculum and its dilution. Throughout the research period, the animals experienced no harm.

The statistical data processing included descriptive statistics employing the statistical analysis package for Microsoft Excel. The significance of differences between mean values was evaluated by Student's t-test. The differences were considered tatistically significant at $P<0.05$.

RESULTS

Cultivation of the probiotic microorganism *B. subtilis* on a grain-based nutrient medium of naked oat (*Avena nuda*) allowed defining the specific growing microorganism. The evaluation of the metabolic activity of a probiotic microorganism revealed that the maximum count reached 4.9×10^{7} CFU/cm^3 on the 6th day of cultivation on the medium based on sprouted avena. On the 10–11th day the number of CFUs has stabilised around $1.9\text{-}2.0\times10^{7}$ CFU/mL.

The experimental liquid probiotic suspension was a bioactive product. Its physical properties were revealed through its specific odour and colour. The dynamics of proteinogenic amino acids correlated with CFU values of probiotic microorganism *B. subtilis.* The analysis of proteinogenic amino acids (Table 1) outlined the culture liquid as an experimental probiotic suspension. It is noteworthy that the weight fraction of crude protein was slightly different in the experimental samples.

It is reasonable to assume that the reduction of the crude protein was possible owing to fermentation of the naked oat grains during germination. The qualitative and quantitative composition of the probiotic suspension featured the complete protein content of the suspension enriched with organic acids. Along with that, the analysis of indicators (Table 1) demonstrated increase in the activity of *B. subtilis* metabolism after cultivation on a non-sprouted avena-based nutrient medium. The synthesis of lysine was higher when compared to the sprouted grain control. In the culture fluid, the amount of amino acids was within the following ranges: lysine from 7.85 to 10.53 g/L, methionine from 2.03 to 2.35 g/L, leucine + isoleucine from 5.79 to 9.7 g/L. A similar trend was observed for other proteinogenic amino acids ($P\leq 0.05$).

When *B. subtilis* was cultivated on a grain-based nutrient medium of nude oat (*Avena nuda*) both non-sprouted and sprouted, EPS (experimental probiotic suspension) was a dietary supplement with

Table 1. Indicators of amino acid composition of the probiotic suspension. Values are presented as mean±SD, n=3

Indicators		Control	Experiment	Control	Experiment
		Avena	*Avena* with *B. subtilis*	Sprouted *Avena*	Sprouted *Avena* with *B. subtilis*
		1	2	3	4
Weight fraction of crude protein, g/L		0.80±0.23	0.73±0.31	0.79±0.29	0.70±0.37
Weight fraction of proteinogenic amino acids, mg/L	Arginine	14.61±0.40	14.40±1.50*	6.55±0.11	3.87±1.25
	Lysine	6.91±0.52	9.80±1.80	5.18±0.25	7.67±1.97
	Tyrosine	10.31±1.10	12.00±0.90*	6.71±1.12	8.93±1.87
	Phenylalanine	8.70±0.90	9.12±0.70*	4.38±1.35	5.60±1.21
	Histidine	5.09±1.30	4.30±1.90	3.45±1.21	2.49±1.37
	Leucine + isoleucine	24.94±0.90	25.19±0.30*	19.04±0.90	15.49±0.50
	Methionine	3.65±1.70	5.81±1.90	3.78±1.25	3.46±1.37
	Valine	11.65±0.70	12.68±0.80*	13.40±0.70	8.72±1.29
	Proline	19.27±0.10	22.37±0.30	22.34±0.90	19.97±0.30
	Threonine	14.97±0.90	10.06±1.70*	15.59±0.20	7.89±1.33
	Serine	20.61±0.90	17.68±1.20*	19.81±0.80	10.83±0.10
	Alanine	15.13±0.50	20.50±1.40	23.13±0.40	20.49±0.80
	Glycine	10.32±1.50	18.20±1.60*	7.84±1.58	8.66±1.33

Note: * statistically significant difference at $P<0.05$ between *Avena* with *B. subtilis* and sprouted *Avena* with *B. subtilis* on the 14th day of the experiment.

probiotic microorganisms at a concentration reaching at least 2.0×10^9 CFU/mL, crude protein content ranging from 0.7 to 0.8 g/L, and proteinogenic amino acids attributing to EPS protein composition.

The experiment with pre-weaning piglets provided background parameters of gastrointestinal microbiocenosis on the 10th day of life. The microbial background in faeces reached 96%, and was made of bifidobacteria and lactobacilli at parity, i.e. averaging about 0.2×10^8 CFU/g. The total *Escherichia* count reached 1.87% or 0.27×10^6 CFU/g (Table 2). Other microorganisms on a standalone basis reached less than 1%. Besides no *Salmonella* or *Candida* yeasts were detected.

On the 25th day an increase in *B. bifidum* was observed in Groups 1 and 2 (Table 2). In Group 2 the count of bifidobacteria in faeces of piglets reached 0.25×10^9 CFU/g, which was by 8% higher than that in Group 1, and by 89.2% higher than in control group. The count of lactobacilli in faeces of piglets from Group 2 reached 0.82×10^8 CFU/g, which was by 14% higher than that in Group 1, and by 36% higher than in Group 3 (control).

Increased count of *Proteus* was also observed, with maximum value of 0.12×10^5 CFU/g recorded in control group: by 16.7% higher vs Group 1, and by 21.7% higher vs Group 2. A similar shift in proportions was observed for other microorganism types: in Groups 1 and 2 their count was significantly lower, which can be attributed to the prominent antago-

Table 2. Dynamics of microbiocenosis formation in piglets (mean ± SD, n=5)

Microorganism type (CFU/g in faecalis)	Group 1 *B. subtilis* in medium of non-sprouted *Avena*			Group 2 *B. subtilis* in medium of sprouted *Avena*			Group 3 Control		
	Days			Days			Days		
	10	25	40	10	25	40	10	25	40
Bifidobacterium	$0.2\times10^{8}\pm 0.29\times10^{8}$	$0.23\times10^{9}\pm 0.14\times10^{9}$	$0.31\times10^{9}\pm 0.61\times10^{9*}$	$0.20\times10^{8}\pm 0.72\times10^{8}$	$0.25\times10^{9}\pm 0.31\times10^{9*}$	$0.36\times10^{9}\pm 0.61\times10^{9*}$	$0.20\times10^{8}\pm 0.24\times10^{8}$	$0.27\times10^{8}\pm 0.93\times10^{8}$	$0.30\times10^{8}\pm 0.14\times10^{8}$
Lactobacillus	$0.23\times10^{8}\pm 0.23\times10^{8}$	$0.71\times10^{8}\pm 0.47\times10^{8}$	$0.8\times10^{8}\pm 0.11\times10^{8*}$	$0.19\times10^{8}\pm 0.06\times10^{8}$	$0.82\times10^{8}\pm 0.91\times10^{8*}$	$0.84\times10^{8}\pm 0.09\times10^{8*}$	$0.2\times10^{8}\pm 0.09\times10^{8}$	$0.53\times10^{8}\pm 0.32\times10^{8}$	$0.78\times10^{8}\pm 0.82\times10^{8}$
Enterobacter	$0.21\times10^{6}\pm 0.52\times10^{6}$	$0.23\times10^{6}\pm 0.28\times10^{6}$	$0.36\times10^{6}\pm 0.31\times10^{6}$	$0.19\times10^{6}\pm 0.16\times10^{6}$	$0.21\times10^{6}\pm 0.38\times10^{6}$	$0.32\times10^{6}\pm 0.52\times10^{6}$	$0.20\times10^{6}\pm 0.67\times10^{6}$	$0.30\times10^{6}\pm 0.44\times10^{6}$	$0.70\times10^{6}\pm 0.38\times10^{6}$
Proteus	$0.65\times10^{4}\pm 0.33\times10^{4}$	$0.10\times10^{5}\pm 0.11\times10^{5}$	$0.47\times10^{5}\pm 0.51\times10^{5}$	$0.73\times10^{4}\pm 0.46\times10^{4}$	$0.94\times10^{4}\pm 0.82\times10^{4}$	$0.40\times10^{5}\pm 0.06\times10^{5}$	$0.70\times10^{4}\pm 0.31\times10^{4}$	$0.12\times10^{5}\pm 0.09\times10^{5}$	$0.30\times10^{7}\pm 0.41\times10^{7}$
Staphylococcus	$0.27\times10^{4}\pm 0.64\times10^{4}$	$0.23\times10^{6}\pm 0.37\times10^{6}$	$0.93\times10^{6}\pm 0.88\times10^{6}$	$0.32\times10^{4}\pm 0.13\times10^{4}$	$0.22\times10^{6}\pm 0.17\times10^{6}$	$0.87\times10^{6}\pm 0.56\times10^{6}$	$0.31\times10^{4}\pm 0.09\times10^{4}$	$0.43\times10^{6}\pm 0.12\times10^{6}$	$0.90\times10^{8}\pm 1.01\times10^{8}$
Clostridium	$0.30\times10^{4}\pm 0.17\times10^{4}$	$0.28\times10^{5}\pm 0.19\times10^{5}$	$0.21\times10^{6}\pm 0.07\times10^{6}$	$0.27\times10^{4}\pm 0.61\times10^{4}$	$0.26\times10^{6}\pm 0.17\times10^{6}$	$0.19\times10^{6}\pm 0.21\times10^{6}$	$0.27\times10^{4}\pm 0.12\times10^{4}$	$0.18\times10^{6}\pm 0.09\times10^{6}$	$0.10\times10^{7}\pm 0.06\times10^{7}$
Escherichia	$0.27\times10^{6}\pm 0.14\times10^{6}$	$0.31\times10^{6}\pm 0.26\times10^{6}$	$0.50\times10^{6}\pm 0.72\times10^{6}$	$0.26\times10^{6}\pm 0.54\times10^{6}$	$0.30\times10^{6}\pm 0.26\times10^{6}$	$0.49\times10^{6}\pm 0.54\times10^{6}$	$0.25\times10^{6}\pm 0.36\times10^{6}$	$0.35\times10^{6}\pm 0.24\times10^{6}$	$0.52\times10^{6}\pm 0.91\times10^{6}$
Salmonella	negative	$0.27\times10^{2}\pm 0.14\times10^{2}$	$0.30\times10^{2}\pm 0.37\times10^{2}$	negative	$0.20\times10^{2}\pm 0.39\times10^{2*}$	$0.23\times10^{2}\pm 0.41\times10^{2}$	negative	$0.11\times10^{5}\pm 0.11\times10^{5}$	$0.17\times10^{5}\pm 0.09\times10^{5}$
Candida	negative	negative	$0.30\times10^{2}\pm 0.29\times10^{2}$	negative	negative	$0.27\times10^{2}\pm 0.47\times10^{2}$	negative	$0.13\times10^{3}\pm 0.21\times10^{3}$	$0.18\times10^{3}\pm 0.16\times10^{3}$

Note: * statistically significant at $P\leq0.05$.

nistic effect of *B. bifidum*. It is noteworthy that on the 25th day, *Salmonella* were found in feces of piglets from all three groups. Their largest count, i.e. 0.11×10^5 CFU/g, was registered in controls, whereas the lowest one – in Group 2, 0.2×10^2 CFU/g, which was by 26% lower than that in Group 1.

On the 40th day in Groups 1 and 2, the largest share of total microbial counts was that of *B. bifidum*: 79% and 80.73% respectively. Lactobacilli reached 0.4% in feces of Group 1, and 18.83% – in Group 2. Other types of microorganisms reached 0.52% and 0.43% respectively.

Staphylococci predominated in the control group: 44.28% of total count. The share of lactobacilli was 38.38%, bifidobacteria were only third with 14.76%. The share of *Proteus* was 1.47%, and that of other types of microorganisms – 1.1% of the total count. When compared to Group 2, the count of bifidobacteria in faeces in piglets from Group 1 was lower by 13.9%. In control group 3, the count of bifidobacteria was lower by 91.7% than in Group 2.

The present research studies contributed to the justification of a reasonable opportunity to use *Avena nuda* as a nutrient medium to produce metabolites during cultivation of the probiotic microorganism *B. subtilis*. Cultivation of the probiotic microorganism *B. subtilis* allowed obtaining a metabolite composition by way of proteinogenic amino acids. The experimental probiotic suspension when *B. subtilis* was cultivated on the grain-based nutrient medium, both non-sprouted and sprouted, was a dietary supplement containing probiotics in an amount of at least 2.0×10^9 CFU/mL

Research studies involving animals proved that the use of the probiotic suspension of *B. subtilis* with a grain-based nutrient of naked oat (*Avena nuda*) in the nutrition of piglets had a significant influence on the proportion of microorganisms in gastrointestinal tract microbiocenosis .

DISCUSSION

The production of dietary supplements based on probiotic microorganisms is based on their metabolites obtained during culturing on different nutrient media. Grain-based nutrient media are the best choice for obtaining biologically active substances from probiotic microorganisms. The outcome of studies in view of *B. subtilis* cultivation are consistent with the focus of the research and with the findings of other authors (Parker, 1974; Nguyen *et al.*, 2016).

However, the production of medicines and dietary supplements based on *B. subtilis* was done in Russia and Germany only in medical practice (Bactistatin and Hylak Forte products). The research studies related to the production and administration of dietary supplements based on metabiotics in animal husbandry and veterinary medicine are at their initial stage of research (Shenderov, 2013; Lebeer *et al.*, 2018).

Several recent research papers (Plotnikova, 2018; Singh *et al.*, 2018) discuss an analogue of the capsulated metabiotic and prove that it can be used to correct microbiocenosis gastrointestinal tract disorders in animals.

In our research studies, *B. bifidum* prevails in formation of microbiocenosis in piglets through metabiotics. The use of probiotic metabolites, according to some authors, not only stimulates the activity of enzyme systems, producing beneficial effect on metabolism in body systems of animals, but also improves the ecological environment of intestine from physiological point of view in order to create and

maintain own microflora, as well as regulates symbiotic relationships with the host (Wang, 2014; Patel & DuPont, 2015; Neschilyaev *et al.*, 2016; Bai *et al.*, 2017).

Published research on *B. subtilis* (Huang, 2013; Orel, 2014; Patel & DuPont, 2015; Ardatskaya *et al.*, 2017; Strekalovskikh & Belousov, 2018) revealed a significant correlation between the content of *Bifidobacterium* and *Lactobacillus,* as well as significant negative correlation between *Enterobacterisceae* and *Clostridium perfingens*. The results from intestinal microflora studies makes it possible to affirm that the use of probiotics influences positively microbial composition: the count of lactobacilli and bifidobacteria increases. There is an established tendency towards reduction in the number of *Escherichia* and staphylococci, and absence of lactose negative *Escherichia*.

The experimental data obtained in these studies are consistent with research data (Pajarillo *et al.*, 2015; Sánchez *et al.*, 2016), confirming the favourable effect of EPS on formation of microbiocenosis in early-weaned piglets. Throughout the research, it was shown that *B. bifidum* was integrated better into microbiome of gastrointestinal tract in piglets of Group 2, which received suspension enriched with probiotic microorganisms grown on sprouted avena medium (Nguyen *et al.*, 2016).

It was demonstrated that the faecal count of bifidobacteria in piglets from this Group was by 13.9% higher than that in Group 1. In controls, bifidobacteria count was by 91.7% lower compared to Group 2, proving the active formation of gastric intestinal microbiocenosis in piglets through direct cultivation of *B. bifidum* in the intestinal tract. The experimental probiotic suspension on the basis of the probiotic microorganism *B. subtilis* helps colonisation of microbiome in normal intestinal microflora, consistent with other studies (LeBlanc, 2014; Ardatskaya, 2015; Fedorova *et al.*, 2016; Sánchez *et al.*, 2016).

CONCLUSION

The administration of grain-based *B. subtilis* probiotic suspension from *Avena nuda* to piglets greatly influenced the proportion of microorganisms in the microbiocenosis composition of the gastrointestinal tract. In piglets treated with an experimental probiotic suspension, the content of *B. bifidum* in faeces was significantly higher than in controls. The microbiome was predominantly represented by bifidobacteria and lactobacilli, with the remaining microflora being less than 1%. A more prominent antagonistic effect was revealed through *B. subtilis* on the medium based on the *Avena* sprouts.

REFERENCES

Ardatskaya, M. D., 2015. Probiotics, prebiotics and metabolics in correction of microecological bowel disorders. *Medical Council*, **13**, 94–99 (RU).

Ardatskaya, M. D., L. G. Stolyarova, E. V. Arkhipova & O. Yu. Filimonova, 2017. Metabiotics as natural development of probiotic conception. *Difficult Patient*, **15**, 35–39 (RU).

Bai, K., Q. Huang, H. J. Zhang, J. Zhang & T. Wang, 2017. Supplemental effects of probiotic *Bacillus subtilis* fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. *Poultry Science*, **96**, 74–82.

Bekhtereva, M. K., A. M. Lukyanova & T. S. Khorosheva, 2014. Modern approaches to rational therapy of bacterial diarrhea. *Lechashchiy Vrach*, **12**, 54–57 (RU).

Belyaev, V., 2020. How Vitacid optimizes the process of digestion in pigs during nursery and fattening. *Swine Rearing*, **4**, 25–26 (RU).

Chae, J. P., E. A. B. Pajarillo, J. K. Oh, H. Kim & D. Kang, 2016. Revealing the combined effects of lactulose and probiotic enterococci on the swine faecal microbiota using 454 pyrosequencing. *Microbial Biotechnology*, **9**, 486–495.

de LeBlanc, A. M. & J. G. LeBlanc, 2014. Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. *World Journal of Gastroenterology*, **20**, 16518–16528.

Fedorova, O. V., A. I. Nazmieva, E. I. Nuretdinova & R. T. Valeeva, 2016. Probiotic products based on microorganisms of the genus *bacillus*. *Journal of the University of Technology*, **19**, 170–174 (RU).

Fisinin, V. I., E. A. Artemieva, I. I. Chebotarev, G. Yu. Laptev, I. N. Nikonov, L. A. Ilyina, N. G. Mashentseva, A. V. Savinov, D. L. Klabukova, E. A. Yildirim & N. I. Novikova, 2017. Dietary probiotic *Lactobacillus plantarum* L-211 for farm animals. II. The additive for piglets. *Agricultural Biology*, **52**, 418–424 (RU).

GOST 32044.1-2012. Feeds, mixed feeds and raw material. Determination of mass fraction of nitrogen and calculation of mass fraction of crude protein. Part 1. Kjeldahl method; https://gostperevod.com/gost-32044-1-2012.html (RU).

GOST R 55569-2013. Feedstuffs, compound feeds, feed raw materials. Determination of proteinogenic amino acids using capillary electrophoresis; https://gostperevod.com/gost-r-55569-2013.html (RU).

Hansen, C., 2018. Focusing on early life piglet performance is critical for success. *International Pig Topics*, **33**, 6–7.

Huang, X.-Z., 2013. Bacterial colonization and intestinal mucosal barrier development. *World Journal of Clinical Pediatrics*, **2**, 46–53.

Kislenko, V. N., 2005. Workshop on Veterinary Microbiology and Immunobiology. Kolos-S (RU).

Kondrakhina, I. P., A. V. Arkhipova & V. I. Levchenko, 2004. Methods of Veterinary Clinical Laboratory Diagnostics: Handbook. Kolos-S (RU).

Lebeer, S., P. A. Bron, M. L. Marco, J.-P. Van Pijkeren, M. O'Connell Motherway, C. Hill, B. Pot, S. Roos & T. Klaenhammer, 2018. Identification of probiotic effector molecules: Present state and future perspectives. *Current Opinion in Biotechnology*, **49**, 217–223.

Lin, L. & Zhang, J., 2017. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunology*, **18**, 2.

Magomedaliyev, I. M., R. V. Nekrasov, M. G. Chabaev, V. V. Dzhavakhiya, E. V. Glagoleva & M. I. Kartashov, 2020. Influence of probiotic complex on productive performance and metabolism in growing pigs. *Agrarian Science*, **1**, 22–26.

Neschilyaev, V. A., P. A. Mokin & T. V. Fedorova, 2016. On development of highly effective metabolite probiotics. *Topical Directions of Scientific Research: From Theory to Practice*, **8**, 15–17.

Nguyen, H.-T., D.-H. Truong, S. Kouhoundé, S. Ly, H. Razafindralambo & F. Delvigne, 2016. Biochemical engineering approaches for increasing viability and functionality of probiotic bacteria. *International Journal of Molecular Sciences*, **17**, 867.

Orel, R., 2014. Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. *World Journal of Gastroenterology*, **20**, 11505–11524.

Pajarillo, E. A. B., J. P., Chae, M. P. Balolong, H. B.Kim, K.-S. Seo & D.-K. Kang, 2015. Characterization of the fecal microbial communities of duroc pigs using 16S rRNA gene pyrosequencing. *Asian-Australasian Journal of Animal Sciences*, **28**, 584–591.

Parker, R. B., 1974. Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health*, **29**, 4–8.

Patel, R. & DuPont, H. L., 2015. New approaches for bacteriotherapy: Prebiotics, new-generation probiotics, and synbiotics. *Clinical Infectious Diseases*, **60** (Suppl. 2), S108–S121.

Plotnikova, E. Yu., 2018. Effects of active *Bacillus subtilis* metabolites in a probiotic product of the new generation. *RMJ Medical Review*, **3**, 39–44 (RU).

Popov, V. S., N. M. Naumov & G. A. Svazlyan, 2020a. Scientific practical justification and aspects of the use of metabolite probiotics in correction of microbiocenosis. *UlGAU Bulletin*, **52**, 198–205 (RU).

Popov, V., N. Vorobyeva, G. Svazlyan, N. Naumov & O. Gryaznova, 2020b. Overview of metabiotics and probiotic cultures during fermentation of molasses. *Systematic Reviews in Pharmacy*, **11**, 813–817.

Potthast, C., S. Brinker & K. Maier, 2011. Futtermittel aus der Zuckerrübenverarbeitung – neue Daten zu Inhaltsstoffen aus einer bundesweiten Erhebung. *Sugar Industry*, **136**, 639–669.

Pritychenko, A. V., A. N. Pritychenko & M. P. Babin, 2012. Intestinal microbiocenosis in detached piglets. *Topical Problems of Intensive Development of Animal Husbandry*, **15**, 258–263.

Roselli, M., R. Pieper, C. Rogel-Gaillard, H. de Vries, M. Bailey, H. Smidt & C. Lauridsen, 2017. Immunomodulating effects of probiotics for microbiota modulation, gut health and disease resistance in pigs. *Animal Feed Science and Technology*, **233**, 104–119.

Rusaleyev, V. S., O. V. Pruntova & D. A. Vasilyev, 2019. Cultural morphological and biochemical characteristics of *Bacillus subtilis* strains. *Veterinary Science Today*, **28**, 58–62.

Sánchez, B., S. Delgado, A. Blanco-Míguez, A. Lourenço, M. Gueimonde & A. Margolles, 2016. Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, **61**, 1600240.

Shenderov, B. A., 2013. Metabiotics: Novel idea or natural development of probiotic conception. *Microbial Ecology in Health & Disease*, **24**, 10.3402/mehd.v24i0. 20399.

Singh, A., V. Vishwakarma & B. Singhal, 2018. Metabiotics: The functional metabolic signatures of probiotics: Current state-of-art and future research priorities – Metabiotics: Probiotics effector molecules. *Advances in Bioscience and Biotechnology*, **9**, 147–189.

Sorokulova, I., 2013. Modern Status and Perspectives of Bacillus Bacteria as Probiotics. *Journal of Probiotics & Health*, **1**, 4.

Strekalovskikh, A. V. & A. I. Belousov, 2018. Scientific rationale for the use of probiotics and metabiotics for prevention of gastrointestinal diseases (literature review). *Youth and Science,* **3**, 30 (RU).

Ushakov, N. A., R. V. Nekrasov, I. V. Pravdin, N. V. Sverchkova, E. I. Kolomeits & D. S. Pavlov, 2015. Mechanisms of influence of probiotics on symbiotic digestion. *Bulletin of the Russian Academy of Sciences. Biological Series*, **5**, 468–476 (RU).

Uyeno, Y., S. Shigemori, & T. Shimosato, 2015. Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environments*, **30**, 126–132.

Wang, Z.-K., 2014. Intestinal microbiota pathogenesis and fecal microbiota transplantation for inflammatory bowel disease. *World Journal of Gastroenterology*, **20**, 14805–14820.

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