STUDYING THE INTERACTION OF PROBIOTIC STRAIN *B. SUBTILIS* AND CONIFEROUS-ENERGY SUPPLEMENT AND THEIR INFLUENCE ON THE MANIFESTATION OF ANTIMICROBIAL PROPERTIES AND BODY WEIGHT ACCUMULATION IN EXPERIMENTAL ANIMALS

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Abstract: The work discusses the influence of the coniferous-energy supplement (further referred to as the CES) on the growth properties of probiotic *B. Subtilis*. The results were assessed visually by the nature of growth: not growing, isolated colonies observed, or confluent growth. The obtained data show that in the range of dosages between 0.25 and 1.0 g, the CES had no inhibitory action on the growth of the culture of strain *B. Subtilis* and even increased the antagonistic properties of B. Subtilis. With increasing the content of the CES in the complex, the bacterial growth was suspended and was inhibited at all. Throughout the study, all animals remained alive. In all groups, the rabbits actively ate the feed, reacted to external stimuli, and showed interest towards people. The combination of strain *B. Subtilis* and the CES made by the LLC STC Khiminvest (Nizhny Novgorod, Russia) showed high antimicrobial properties (in the *in vitro* tests), and a positive trend in the dynamics of the body weight growth in the laboratory animals.

Keywords: antimicrobial properties, coniferous-energy supplement (CES), laboratory animals, strain *B. Subtilis*.

1 Introduction

Currently, due to the threat of the development of bacteria resistance to antibiotics, the World Health Organization (WHO) recommends limiting their use in agriculture. Antibiotics received by animals in feed additives circulate in the organism for a long time, and their residual quantities are found in food products of animal origin (milk, eggs, meat) [1]. Eating such food products may be compared to uncontrolled antibiotic therapy, which results in the formation of antibiotic resistance in the pathogens, and, consequently, in dysbiotic and immunosuppressive disorders in the human organism [2].

Animal breeders widely use antibiotics for preventing and treating epizootic diseases, stimulating the growth of the animals, improving the feed quality, and preserving it for business purposes [3].

The RF legislation has established the standards for the content of the most widely used antibiotics in milk and meat, and the products of processing them, including poultry meat, eggs, fish, and seafood [4]. However, the range of the preparations used in the food industry is constantly extending, therefore, the content of many of them in food products remains not normalized. The existing monitoring measures cannot determine the content of all used antibiotics.

These circumstances indicate the need for developing the methods for monitoring antibiotics and for searching for efficient alternatives to antibiotics. One such area is the development of new pre- and probiotic preparations [5]. Prevention and treatment of infections in farm animals with efficient bio preparations is a more physiological and safe approach to obtaining high-quality and environmentally friendly products.

In the world of veterinary practice, preparations created based on probiotic strains of bacteria of genus *Bacillus* have become widely used [6]. They are safe for the macroorganisms even in high concentrations (except for *B. Anthracis* and *B. Cereus*), produce antibiotics (bacitracin, bacilizin, bacillomicin, bacillin, gramicidin, iturin, obutin, proticin, petrin, subtilin, doximycin, trypanotoxin, fluvomycin, endosubtilysin), enzymes of various classes (hydrolases, oxidoreductases, transferases, lyases, lygases), amino acids (alanine, valine, isoleucine, leucine, lysine, methionine, etc.), vitamins (B6, B12, riboflavin, thiamin, nicotinic and pantothenic acids); they are technologically simple in production and stable during storage [7, 8]. Choosing the most functional strains of the bacteria of genus *Bacillus* will allow creating efficient biological products for livestock breeding, and to abandon the use of antibiotics. In this aspect, strain *B. Subtilis* obtained at the microbiological laboratory of the SRIVS n.a. I. I. Mechnikov is of great interest. The strain shows a broad spectrum of antagonistic activities against opportunistic pathogenic bacteria and fungi, low adhesiveness, immunomodulatory properties; it produces hydrolytic enzymes that help improve the digestive processes [9].

For normalizing the physiological processes in animals and for improving their growth and productivity, feed additives based on recycled forest biomass are also currently used. They are inexpensive, environmentally clean, and safe [10, 11]. The CES made at the LLC STC Khiminvest (Nizhny Novgorod, Russia) is used as a feed supplement and is intended for the replenishment of nutrients and energy in the diet of agricultural animals. [12, 13]. It makes the organisms of farm animals saturated with water-soluble vitamins (C, B1, B6, PP, etc.), fat-soluble vitamins (A, E, D, etc.), carbohydrates (glucose, fructose, galactose, sucrose, maltose), minerals (phosphorus, calcium, iron, magnesium, etc.), and amino acids (arginine, methionine, lysine, etc.) [14, 15].

Based on the identified properties of strain *Bacillus Subtilis* and the CES, studying their mutual influence and expediency of developing a veterinary biological product is of great interest.

This work was aimed at studying the interaction of the probiotic strain and the coniferous supplements and their influence on the manifestation of antimicrobial properties and body weight gain in laboratory animals.

2 Materials and methods

The material for the study was the CES made at the LLC STC Khiminvest (Nizhny Novgorod) and the biomass of probiotic strain *Bacillus Subtilis*.

The antagonistic activity was determined by the method of deferred antagonism [16] relative to the test strains of opportunistic pathogenic bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* 9022, *Staphylococcus aureus* 29213, *Shigella Sonnei* 170, *Proteus mirabilis* 24a from the collection of the FSBEI SRIVS n.a. I. I. Mechnikov.

In the experiments, 20 female rabbits of the Soviet chinchilla breed with a weight of 2 – 2.5 kg were used. The monitored parameters were survival rate, appearance, behavior, and the dynamics of body weight gain.

The animals were divided into four groups five individuals in each, which within 60 days received orally the following: Group

1 — *B. Subtilis* at the dosage of 1 * 10^9; Group 2 — the CES at the dosage of 1 g; Group 3 — *B. Subtilis* at the dose of 1 * 10^9 and the CES at the dosage of 1 g (further referred to as "the Complex"); and group 4 — reference. To study the dynamics of weight change, the animals were weighed weekly for two months.

3 Results and discussion

At the initial stage, the influence of various dosages of the CES (0.25 to 7.5 g) on the growth properties of *B. Subtilis 1719* was studied (Table 1). For this purpose, nutrient agar and the CES were poured into Petri dishes and gently stirred. *B. Subtilis* was planted on the solidified agar, and the biomass was spread on the surface with a spatula. The cups were incubated in a thermostat at 37 ^oC for 24 hours. The results were assessed visually by the nature of growth: not growing, isolated colonies observed, or confluent growth. For the reference, the CES was not added to one dish.

Table 1. CES interaction with strain *B. Subtilis*

No.	Nutrient medium	CES	*B. Subtilis*	Result
	ml	g	ml	
1	20	7.5	0.1	Not growing
2	20	5	0.1	Isolated colonies are observed
3	20	2.5	0.1	Isolated colonies are observed
4	20	1	0.1	Confluent growth
5	20	0.5	0.1	Confluent growth
6	20	0.25	0.1	Confluent growth
7	20	-	0.1	Confluent growth

The obtained data showed that in the range of dosages between 0.25 and 1.0 g, the CES had no inhibitory action on the growth of the culture of strain *B. Subtilis*. With increasing the content of CES in the complex, the bacterial growth was suspended and inhibited at all.

The antagonistic activity of the complex preparation against opportunistic pathogenic bacteria was assessed in comparison with monostrain *B. Subtilis (*Table 2).

Table 2. Antagonistic activity of strain *B. Subtilis* and the studied complex against opportunistic pathogenic bacteria

The name of the test culture		Test cultures growth inhibition, mm (M ± m)	
		B. Subtilis	Complex
1	Escherichia coli ATCC 25922	19 ± 1.5	21 ± 2.5
2	Pseudomonas aeruginosa 9022	21 ± 2.5	22 ± 1
3	Staphylococcus aureus 29213	18 ± 2.5	21 ± 1
4	Shigella Sonnei 170	19 ± 2	20 ± 3
5	Proteus mirabilis 24a	16 ± 1.5	18 ± 2.5

The obtained data testified that the source probiotic strain *B. Subtilis* had high antagonistic activity against opportunistic pathogenic bacteria. It was also evident that the CES, due to its properties, enhanced the antagonistic properties of *B. Subtilis*.

Throughout the study, all animals remained alive. In all groups, the rabbits actively ate the feed, reacted to external stimuli, and showed interest towards people. The results of weighing the rabbits are shown in Table 3 (the average result per group). The rabbits' body weight had positive dynamics in all groups.

Table 3. The dynamics of rabbits' body weight (kg)

Time, days	Group of animals that received			
	Group 1 *B. Subtilis*	Group 2 CES	Group 3 Complex	Group 4 Reference
1	2.61	2.44	2.68	2.41
8	2.68	2.52	2.75	2.49
15	2.74	2.60	2.88	2.53
22	2.88	2.74	3.04	2.55
29	3.02	2.81	3.24	2.69
36	3.19	2.97	3.37	2.79
43	3.18	2.99	3.49	2.87
50	3.34	3.18	3.63	2.97
57	3.40	3.28	3.80	3.08
61	3.48	3.31	3.83	3.15

Table 4. The dynamics of rabbits' body weight (%)

Time, days	Group of animals that received			
	Group 1 *B. Subtilis*	Group 2 CES	Group 3 Complex	Group 4 Reference
61	+ 33.33	+ 35.66	+ 42.91	+ 30.71

Table 4 shows that the use of veterinary preparation ensures the highest live weight gain in the animals, compared to the reference group.

4 Conclusion

Thus, the combination of a probiotic strain of *B. Subtilis* and the CES made by the LLC STC Khiminvest (Nizhny Novgorod, Russia) has shown high antimicrobial properties (in the *in vitro* tests), and a positive trend in the dynamics of the body weight growth in the laboratory animals. This combination may be recommended for further study as a symbiotic veterinary biological product.

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