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Effect of new probiotic of spore-forming *Bacillus Subtilis,* cultivated on a local agro-industrial waste, on rabbit productivity and meat quality

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Abstract

The aim of the research was to develop a low-cost, innovative and competitive methodology of production of spore-forming probiotics, demonstrating effectiveness as an alternative to antibiotics, by controlling and preventing gastrointestinal diseases in rabbits, improving their productivity and the quality of manufactured products, achieving economic well-being.

A number of scientific and technological tasks were implemented in order to achieve this goal.

Probiotic preparations and confirmation of their best effect was carried out in the rabbit enterprise “Organic Solution" LLC.

As a result of the conducted 6 experiments, it was determined that the optimal dose of adding new spore-forming *Bacillus subtilis* probiotics to the feed of the rabbit is 0.04%.

In the spore-forming probiotic material made from local agro-industrial raw materials, the spores retain their viability to an equal degree, so their storage in dry ground form is important from the point of view of practical use;

The number of spores of the spore-forming probiotics of the fresh preparation mixed with granulated feed and stored for 6 months is unchanged and successfully maintains its activity.

The research was carried out with the financial support of the Shota Rustaveli National Science Foundation (grant project CARYS-19-2268)

Keywords: Rabbit; Antibiotic; Probiotic; Effectiveness; Experiment; Optimal Dose; *Bacillus Subtilis*

1. Introduction

1.1. The relevance of the issue

In recent years, due to the growing demand of rabbit meat in Georgia, the number and the capacity of rabbit farming enterprises has increased significantly. Traditionally, tetracycline, amciloxine, penicillin, bacitracin and other antibiotics are used as preventive antimicrobials and growth stimulants in the production of meat [1]. The use of antibiotics, in turn, has led not only to the emergence of pathogens resistant to multi-antibiotic drugs, but also to a decrease in the necessary microflora and immune function in the plant [2], resulting in production losses and an

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increase in overall costs [3]. The potential transfer of antibiotic resistance from animals to humans has also penetrated [4]. Because of this problem, the use of antibiotics as growth promoters has been restricted in Europe and other developed countries [5]. Therefore, it became mandatory to replace antibiotics with other effective means. The use of probiotics, as microorganisms forming a natural protective barrier between the animal body and pathogens, has become the most realistic, natural alternative to traditional gastrointestinal antibiotic therapy[6].

Traditionally, Lactobacillus , Bifidobacterium , Enterococcus strains dominate probiotics. Lately, spore-forming Bacillus species is widespread in livestock and animal husbandry [8]. Moreover, spore-forming probiotics are produced and used in humans as harmless supplements[9] (eg, Bactisubtil , France; Nature's First Food , USA), as well as in animals as a growth stimulator (for example, BioGrow, Great Britain, Japan). It is also used to increase the growth and resistance of aqua cultures [10] (eg, Biostart , USA; Promarine , Belgium).

It should be noted that *the Bacillus* species has many important technological advantages. These organisms are characterized by high adaptation to environmental conditions and high growth rate on vegetable raw materials. Thermoresistant spores are stable under long-term storage conditions without refrigeration. Moreover, spores have the ability to survive in the gastrointestinal tract even at low pH [11] [12] [13]. Thus, the full dose of bacteria introduced as spores reaches the small intestine unchanged, which is not the case with all species of Lactobacillus [14][15]. In addition, the secretion of antimicrobial compounds (coagulin , amicoumacin and subtilisin) provides a probiotic effect by inhibiting the growth of both competing bacteria and enteric pathogens. *Bacillus* vegetative forms of the species produce extracellular enzymes (protease , cellulase , xylanase , pectanase and lipase) that help in the assimilation and absorption of nutrients[16]. Finally in rabbits the use of probiotics has made it possible to improve the following parameters: 1. The rate of maintenance, reducing their morbidity and mortality 2) Daily weight gain and live mass 3) Feed absorption and conversion [17] 4) Economic efficiency [18] [19][20] .

Based on the mentioned circumstances, the search for alternative methods to remove the antibiotic in the rabbits, its testing and the implementation of the obtained results in practice, is the most important issue of the recent scientific researches in rabbit husbandry.

1.2. The purpose and objectives of the research

In treatment of gastro -intestinal diseases, as mentioned above, farms still use antibiotics as a feed additive. In order to find alternative methods and taking into account the benefits of using the spore-forming probiotic described above, the local market requirements, the lack of production of probiotics in the South Caucasus and the availability of the necessary resources, the team of the Agrarian University of Georgia, with the financial support of the Shota Rustaveli National Science Foundation (project AR/106/7-280/14, 2015-2017), implemented Studies that allowed us to investigate the physiological mechanisms that regulate and improve *Bacillus* probiotic intake by species.

The scientific and technological idea had a dual purpose: to utilize free agro-industrial waste, to process and use the enzyme system and biosynthetic potential of bacteria, to develop an innovative, low-cost and competitive technology of spore cultivation for the production of probiotics. Probiotic made in this way put on the agenda the issue of studying spore-forming *Bacillus subtilis*, a new probiotic, as an antibiotic replacement, feed additive in the nutrition of rabbits, which was the basis for the research.

The testing of the probiotic preparation, confirmation of the best effect, was carried out in the enterprise of “Organic Solution LLC”. For the first time was evaluated *Bacillus subtilis* biological efficiency, feed conversion and productivity of a new probiotic preparation. The research was carried out on several experimental and control groups.

2. Research material and methods

Spore-forming *Bacillus subtilis*, a new probiotic, new strong strains of *Bacillus subtilis* with higher antimicrobial and probiotic activity [21] were selected in the laboratory of Agricultural University of Georgia, to study the effectiveness of the new probiotic, new areas for cultivation with a unique composition were created. One of the world's most productive laboratory technology of solid-phase fermentations for spore production has been developed, which was tested using different plant raw materials [22][23][24]. Solid state fermentation was carried out in climatic cells using polypropylene bags, in which the substrate was placed. Compared to deep fermentation, solid-state fermentation is characterized by less complexity and investment of equipment, increased biomass productivity and less waste generation. After the end of the growth phase, the number of spores were measured in the samples, it was absolutely in line with the planed task. The dry product was ground to obtain a homogeneous loose mass. The spore content of the obtained sample was again measured and packed in paper bags. Testing of the received probiotic and confirmation of its effect was carried out by

animal husbandry and veterinary specialists of the Agricultural University of Georgia. Rabbits were fed with complete feed, the zootechnical analysis of which was carried out at the accredited laboratory "Etalon" LLC. During the test period, three experimental and one control group's were provided with the same environmental and hygienic conditions. For the test group *Bacillus subtilis* probiotic was mixed with the feed at the required concentration using a rotary mixer (0.03%, 0.04% and 0.05% in the finished feed). Whereas, rabbits in the control group received the basic feed along with a standard dose of antibiotics.

3. Research results in production conditions

The testing of *B. subtilis* probiotics was carried out in " Organic" LLC At the "Solution" rabbit enterprise, 30 day-old rabbits were tested, which before (28 day) were fed on mother's milk. From day 30, they were fed with full-fledged balanced feed. During the trial period, rabbits were fed with complete balanced granulated combined feed. Considering the age: up to 1-2 months and up to 2-3 months.

The analysis of the combined feed of 1-2 month old rabbits showed that the crude protein content is 17.5%, and the energy 273 kcal.

The complete combined feed of 2-3 month old rabbits has a crude protein content of 17.2% and energy of 278%.

Thus, the content of nutrients in the feed portion is the same for all experimental and control groups and fully meets the nutritional needs of the Californian breed in both age periods.

The trial was conducted according to the following scheme:

- I group -control, whose basic combined feed was not supplemented with probiotics and periodically received the antibiotic Ernafloxacin together with water .
- II- group (experimental), the main full-fledged combined feed of rabbits, from 30 days to slaughter, was given fresh *B. subtilis* probiotic at a dose of 0.05% and did not receive an antibiotic.
- Group III (experimental) rabbits also did not receive antibiotics, fresh *B. subtilis* Probiotic in the amount of 0.04% was added to their feed daily.
- Group IV (experimental) rabbits did not receive antibiotics and new *B. subtilis* Probiotic in the amount of 0.03% was added to their feed daily.

The new probiotics of spore-forming Bacillus, which replace antibiotics, were used in the feeding of rabbits according to the following scheme in (Table 1).

Table 1 Feeding Scheme with the new probiotics of spore-forming Bacillus

Group	Feed	Probiotic / antibiotic	Quantity Piece
I group (control)	Combined feed with an antibiotic	Ernafloxatsilin	100
Group ii	Combined feed with probiotics	*B. subtilis* 10^8 spore/gr 0.05 %	100
Group iii	Combined feed with probiotics	*B. subtilis* 10^7 spores/gr 0.04 %	100
Group iv	Combined feed with probiotics	*B. subtilis* 10^6 spore/gr 0.03 %	100

During the test period, rabbits were kept in cages. The technological parameters of storage were the same for all groups and fully corresponded to the growing technological requirements. The experimental and control groups were recruited according to the principle of analogues.

At the age of 30 days, the live weight of the rabbits was almost equal in all groups and corresponded to the requirements characteristic of the California breed variety.

During the trial period, it was revealed that in all age periods, the third group had the highest live mass, and the control group had the lowest. At the time of slaughter, the live weight of the rabitts in third group amounted to 2517g, which is 4.2% higher than in control group (Figure 1).

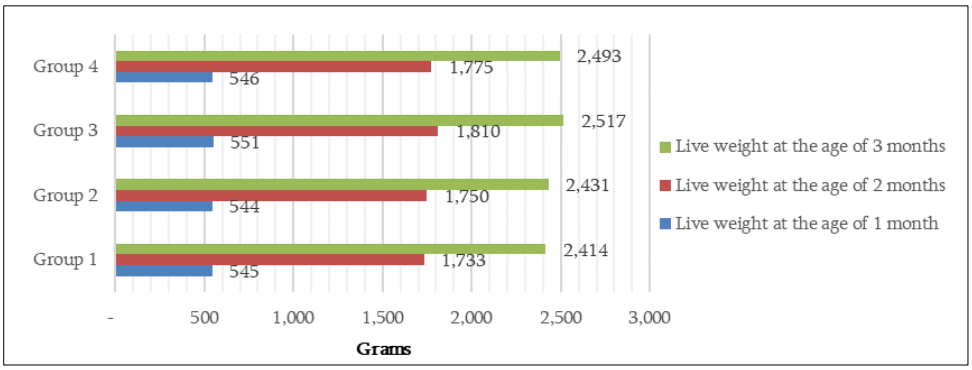

Figure 1 Live weight of rabbits in groups

The calculation of the absolute weight gain during the growing period (30-90 days) showed that the highest absolute weight gain of the third group was 2266 g, which was 21.2% higher than in control group, and the second and fourth groups had 16-20% (Figure 2).

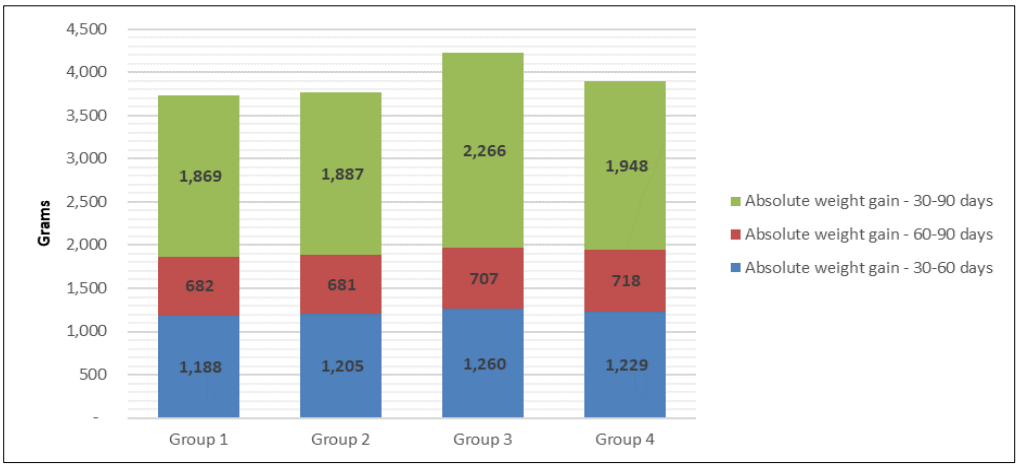

Figure 2 Absolute weight gain of rabbits in groups

The calculation of daily weight gain showed that this indicator was the highest -37.76 g in the third test group (Figure 3).

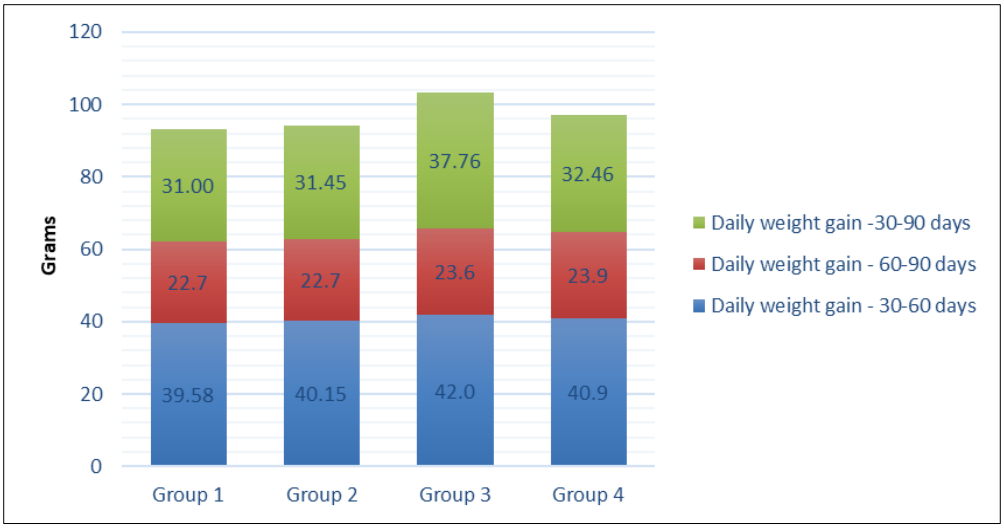

Figure 3 Daily weight gain in groups

During the growing period (30-90 days), the percentage of maintenance in the third group was 95%, and in the control group it was 91% (Table 2). Although rabbits in the control group received periodic antibiotics to prevent gastrointestinal disorder, 5 (56%) out of 9 collapsed of gastrointestinal disorder. This was not observed in the experimental groups where the cause of death was injuries (Figure 4).

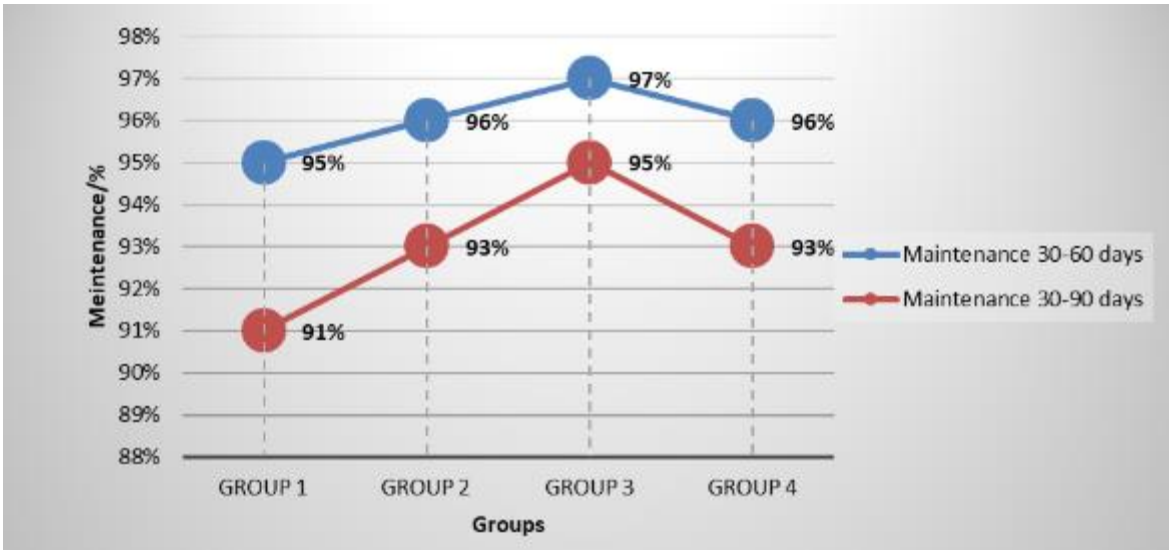

Figure 4 Maintenance rate in groups

The calculation of feed consumption showed us that the feed consumption per animal in all groups during the rearing period was almost the same at 10.92-11.37 kg. The productivity index was the highest in the third test group (Figure 5).

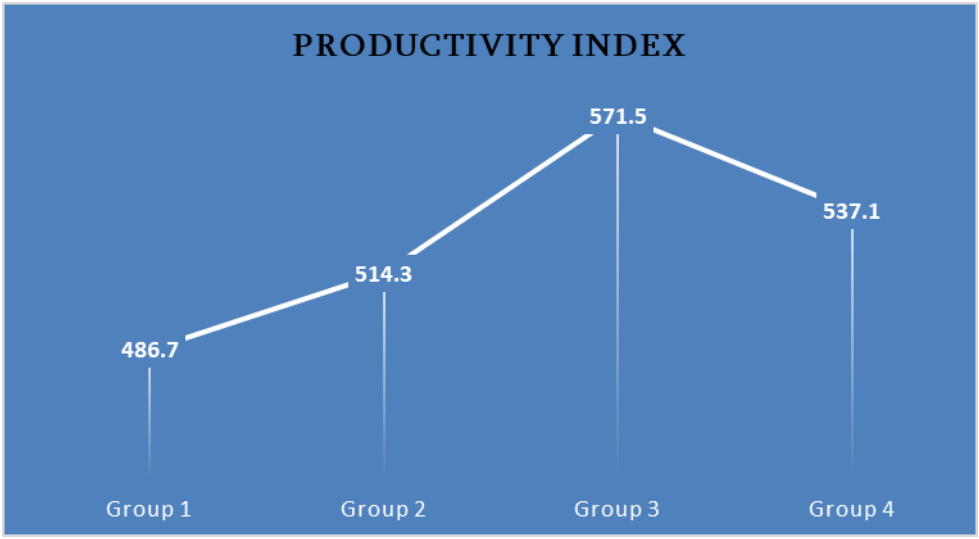

Figure 5 Productivity Index in groups

In the experimental groups, the yield was 56.3-57.2%, which was significantly higher than in control group, which was significantly higher than in control group. In the third experimental group, 83.3% of the meat of the rabbits was in the first category, while only 66.6% of the control group's rabbits were in the first category and 33.4% in the second category. (Table 2)

During the control slaughter, we studied the chemical composition of the meat, at the accredited laboratory of "Expertiza+" LLC, where the water content, mass fraction of protein, fat and ash in the meat were studied, Table 3.

Table 2 Results of the control slaughtering

Indicators	Units of measurement	Groups			
		1	2	3	4
live mass at slaughter	gram	2393	2444	2508	2430
The mass of the carcass	gram	1340	1375	1460	1390
	%	55.98	56.3	58,21	57,20
First category	%	66.6	75.0	83.3	75.0
Second category	%	33.4	25.0	16.7	25.0

Table 3 Chemical composition of the meat

Indicators	Units of measurement	Groups			
		1	2	3	4
mass fraction of moisture	%	77.55	75.67	75.0	76.30
share of total ash	%	1.25	1.25	1.26	1.23
share of fat	%	0.73	1.89	0.89	1.19
share of protein	%	20.31	21.06	21.56	21.13

As the analyzes showed, the water content was found to be the lowest in the meat of the third group - 75%, and the highest in the meat of the control group - 77.55%. The massive share of protein in the meat of the third group was 21.56% and is 1.2% higher than in the control (20.31%). The second and fourth groups also had a higher mass fraction of protein compared to the control. As for fat, this figure was the highest in the second group at 1.84% and in the fourth group at 1.19%. The mass share of total ash in all four groups is almost the same and is 1.23-1.26%.

At the end of rearing, at the age of 90 days, some morphological and biochemical parameters of the blood were examined at the "New Veterinary Clinic" LLC, (Table 4).

Table 4 Rabbit blood morphological Indicators

Indicators	Size unit	Groups			
		1	2	3	4
Hemoglobin HGB	g/l	90	99	111	103
Ethrocytes RBC	10^{12} l	4.5	4.8	5.5	5.0
hematocrit	%	28	31	34	33
Average volume of erythrocytes	mcg ^{3}	52	59	62	59
Platelets PTL	10^9 l	293	440	257	560
Leukocytes WBC	10^9 l	5.0	6.5	6,6	4.8
Eosinophils EOS	%	4.0	4.5	2.5	4.5
Basophils	%	1	1	1	1.5
Monocytes MON	%	4.5	4.5	4.5	7.5
Lymphocytes LYM	%	35	45	37.5	32
Erythrocyte sedimentation rate ESR	mm / h	3.5	2.5	2.5	2.5

Morphological analysis of blood showed that the morphological indicators of blood in all groups are within the norm. However, it should be noted that among the morphological indicators of blood, hemoglobin, the number of erythrocytes and their average volume, hematocrit , were the highest in the third group, and the lowest in the control group (Table 4).

As for some biochemical indicators of blood, the indicators studied by us were within the normal range in all groups (Table 5).

Table 5 Rabbit Blood Biochemical parameters

Indicators	**Groups**			
	1	2	3	4
Alanine aminotransferase	57	55	58	56
Aspartate aminotransferase	68	83	77	83
Gamma Glutamyltransferase	10	10	8	9.5
Total protein, g /l	63	53	58	55
Albumin, g/l	36	34	33	25
globulin g/l	25	19	27	30
Creatinine , micromol /l	133	93	95	63
Urea mol/l	4.2	5.4	4.2	4.8

The effect of a new *B. subtilis* probiotic on the microflora of the gastrointestinal tract of rabbits at the age of 90 days was studied. From each group, 12-12 units (6 females, 6 males) were slaughtered, the rectum was removed along with the feces, and the examination was carried out in an accredited and reviewed laboratory "SANA". The results of the analysis showed that the species composition of the intestinal microflora of rabbits in all four groups is the same and no pathological forms were observed.

4. Conclusion

The optimal dose of the new spore-forming probiotic *Bacillus Subtilis*, made on local agro-industrial raw materials, as a food additive in the feed of rabbits, was determined - 0.04%.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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