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Impact of Spore Forming New Probiotic of Bacillus Subtilis and Bacillus Amyloliquefaciens on Broiler Productivity

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Abstract- **The problem of avian dysbacteriosis is a challenge in the poultry industry. To solve the problem, appropriate antibiotics are used. However, the widespread concern is the appearance of pathogenic microorganisms that are resistant to modern antibiotics. The growing demand to improve the quality of poultry products has put on the agenda the search for alternative methods to replace antibiotics in poultry products. To achieve this goal, the strain of Bacillus subtilis Katmira 1933 exhibiting exceptional probiotic potential has been tested in broilers as a feed additive. B. subtilis and Bacillus amyloliquefaciens were cultivated in solid-state fermentation of local agro-industrial raw materials (wheat bran and vinasse), then dried fermented bioweight containing 1 x 1012 CFU/g was used at a concentration of 0.03%, 0.04%, and 0.05% as a feed additive in the broiler farm "Roster". In parallel with three above-mentioned test groups of birds, the control group was treated with an antibiotic commonly used on a poultry farm. Both control and experimental groups of broilers were fed by a complete combined feed, which met the broiler's demand for nutrients, minerals, and biologically active substances, according to the phases of broiler development.**

Based on the experimentს, it was found that the optimal dose of probiotic B. subtilis, cultivated on plant raw materials, used as a feed additive in broiler is 0.04%. Under these conditions, feed conversion ratio was almost the same in both groups. In the experimental groups, the average daily weight gain during the rearing period was 3.5-3.7 g higher than in the control groups (on average, 53.0-53.2 g/day), the absolute gain in live weight of the experimental broilers increased by 7.3%. Survival rate of experimental groups was 96-98% which is 2-4% higher than in the control group.

Index Terms- **Broiler, antibiotic, probiotic, probiotic efficacy, experiment, optimal dose of probiotic, Bacillus.**

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I. INTRODUCTION

Broiler meat plays an important role in human nutrition. Due to the growing demand for this product in recent years, both in the world and in Georgia, the number of poultry enterprises and their capacity have increased significantly. Traditionally in the production of broiler meat tetracycline, amoxicillin, penicillin, bacitracin and other antibiotics are used as a preventive antimicrobial and growth stimulant. However, the use of antibiotics has led to increase in total costs by 10-15%. Potential transfers of antibiotic resistance from animals to humans have been introduced. Due to this problem, the use of antibiotics as growth stimulants has been banned in Europe and other developed countries. It has therefore become mandatory to replace antibiotics with other effective means. The use of probiotics as microorganisms that create a natural protective barrier between the animal organism and pathogens has become the most realistic natural alternative to traditional gastrointestinal antibiotic therapy, which promotes animal productivity and product safety.

Traditionally, Lactobacillus, Bifidobacterium, Enterococcus strains have dominated probiotics. Recently, the use of spore forming Bacillus species has become widespread in livestock and poultry. Moreover, spore forming probiotics are produced and used in humans as harmless additives (e.g., Bactisubtil, France; Nature's First Food, USA), as well as in animals as growth stimulants (e.g., BioGrow, UK, Japan), it is also used in aqua cultures to increase growth and disease resistance (e.g., 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 rates on plant raw materials. Thermoresistant spores are stable under long-term storage without refrigeration. Thus, the full dose of bacteria introduced in the form of spores invariably reaches the small intestine, which does not occur in all species of Lactobacillus (Tuohy et al., 2007). In addition, the secretion of antimicrobial compounds (coagulin, amikoumacin, and subtilizine) provides a probiotic effect by inhibiting the growth of both competing bacteria and enteric pathogens. Also, vegetative forms of Bacillus species produce extracellular enzymes (protease, cellulase, xylanase, pectanase, and lipase) that promote nutrient digestion and absorption (Chen et al., 2009). Finally, the use of probiotics in poultry has made it possible to improve: 1. Poultry retention rates to reduce their mortality, 2) Daily weight and live weight 3) Food digestion and conversion, 4) Economic efficiency (Kurtoglu et al., 2004; Panda ., 2008).

Taking in to account the above mentioned circumstances, searching for alternative methods to remove the antibiotic load in the avian organism, then testing it and putting the results into practice has been a major issue for ongoing scientific research in poultry industry in recent years.

II. Testing material and methodology

In order to study the effectiveness of new spore forming Bacillus Subtilis and Bacillus amyloliquefaciens probiotics, new strong strains with superior antimicrobial and probiotic action were selected in the laboratory of the Georgian Agrarian University (AlGburi et al., 2016). New nutrient cultivation areas with unique composition have been developed, laboratory technology for the production of one of the highest yield spores in the world, using solid-state fermentation (SSF), which has been tested using a variety of plant raw materials. (Khardziani et al., 2017a; 2017b; Elisashvili et al., 2018). Solid state fermentation was carried out in climate cells using polypropylene bags in which the substrate was placed. Solid state fermentation is characterized by less complexity and requires less investment, increased biomass productivity, and less waste generation compared to deep fermentation. The number of spores in the samples taken at the end of the growth phase was measured; it was consistent with the set task. The dry product was grounded to obtain a homogeneous weight. The spore content was again measured in the sample and packed in paper bags. Veterinary specialists of the Agrarian University in the industrial environment tested the obtained probiotics, at the poultry enterprise “Roster” Ltd., confirmed their positive impact. The broiler was fed according to phases with full-fledged feed, the zootechnical analysis of which was carried out in the accredited laboratory "Etalon" Ltd. During the experiments, three experimental and one control group of birds were provided with the same environment and hygienic conditions. For the experimental group broiler, probiotics of Bacillus Subtilis and Bacillus amyloliquefaciens were mixed separately with feed at the required concentration using a rotary mixer (0.03%, 0.04% and 0.05% in prepared feed). While, the control group birds received basic food along with the standard dose of antibiotic. In order to study the effectiveness of new probiotic drugs, new strong strains with superior antimicrobial and probiotic action were selected in the laboratory of the Georgian Agrarian University (AlGburi et al., 2016). New nutrient cultivation areas with unique composition have been developed, laboratory technology for the production of one of the highest productive spores in the world, solid-state fermentation (SSF), which has been tested using a variety of plant raw materials. (Khardziani et al., 2017a; 2017b; Elisashvili et al., 2018). Solid-phase fermentation was carried out in climate cells using polypropylene bags in which the substrate was placed. Solid-phase fermentation is characterized by less complexity and less investment, increased bioweight productivity, and less waste generation compared to deep fermentation. The number of spores in the samples taken at the end of the growth phase was measured, it was absolutely consistent with the set task. The dry product was ground to obtain a homogeneous loose weight. The spore content was again measured in the sample and packed in paper bags. The obtained probiotics were tested and their influence was confirmed by the livestock and veterinary specialists of the Agrarian University in the production environment, in the poultry enterprise "Roster" Ltd. The broiler will be fed phased whole food, the zootechnical analysis of which will be carried out in the accredited "Etalon" Ltd. During the experiments, three experimental and one control group birds were provided with the same environment and hygienic conditions. For the experimental group broiler, probiotics of Bacillus Subtilis and Bacillus amyloliquefaciens were mixed separately with feed at the required concentration using a rotary mixer (0.03%, 0.04% and 0.05% in prepared feed). While, the control group birds received basic food along with the standard dose of antibiotic.

III. Research results on industrial level

The experiments were conducted at a broiler plant where the broiler feeding was implemented in phases: start 1-10 days, grower 11-28 days and finish 29-35 days. Combined feed was prepared for the control and experimental group birds in "Roster" Ltd. In order to apply new spore forming Bacillus subtilis and Bacillus amyloliquefaciens and to determine the optimal dose for experiments in "Roster" Ltd. In each experiment participated 400-400 oneday birds, 100 birds in group.

Each experiment lasted 35 days. During the experiment we studied: broiler live weight dynamics, individual weighing of poultry was undertaken at 1, 14, 28 and 35 days, absolute and daily increments, maintenance, feed intake during rearing per 1 bird and feed conversion per 1 kg weight, meat output, meat category, morphological and biochemical parameters of the blood, a broiler growth efficiency index.

In both experiments, chemical analysis of the feed showed that the starting protein content in the starter food was 23.6%, energy 305 kcal, raw protein content in the Grower period 20.5%, energy 309 kcal, and in the finish period 18.5% and 316 kcal, respectively. The content of other nutrients was also within the norm and fully met the requirements of the broiler "Ross-308" for nutrients in all ages

Bacillus subtilis application scheme is as follows

Table 1 Experiment scheme:

Group	Feed	Probiotic/Antibiotics	Quantity
Stage I			
I Group (control)	Combined feed with Antibiotic	Ernafloxacillin	100
II Group	Combined feed with Probiotic	B. subtilis 10^{12} spore/gr 0.05%	100
III Group	Combined feed with Probiotic	B. subtilis 10^{12} spore/gr 0.04%	100
IV Group	Combined feed with Probiotic	B. subtilis 10^{12} spore/gr 0.03%	100

The growth dynamics of the broiler during the experiment:

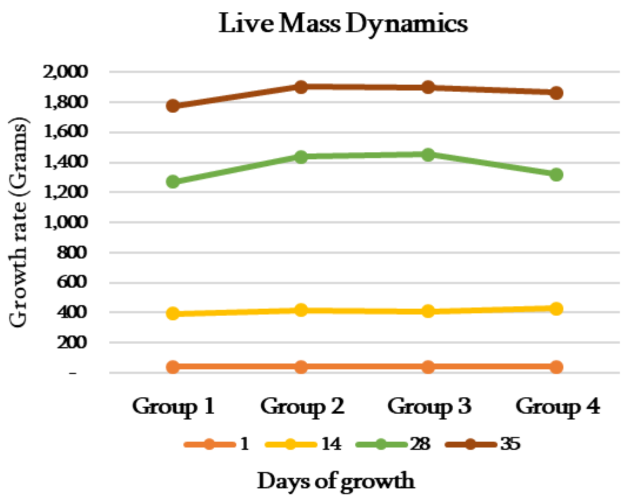

Fig.1. Live mass growth dynamic

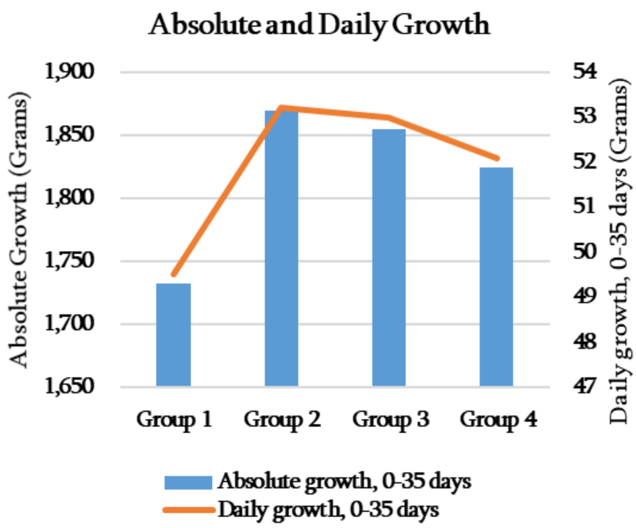

Fig:2 Absolute and daily increase of broiler weight

Fig.1. The live weight of a one-day trial broiler is the same in all four groups of 40.7-40.8 g, which indicates a high uniformity of the test chickens. The live weight of the control broiler at age of 14 days was 395 g, and the broiler weight of the experimental groups with application of new probiotic Bacillus subtilis from day one was 408.4-427.2 g, which was 3.4-8.1% higher compared to the control group ($P \geq 0.01$-0,001). At age of 28 days, the live weight of the experimental group broiler wighted 1322-1452 g, which is 4.0-14.3% higher than the control group ($P \geq 0.01$-0,001). The broiler of the 3rd experimental group had the highest live weight during this period - 1452 g, which is 14.3% higher ($P \geq 0{,}001$) than the broiler live weight of the control group and 9.83% higher ($P \geq 0.01$) higher than the live weight of 4th experimental group. At the age of 35 days, at the end of growth period, the highest live weight was observed in the 2nd experimental broiler group - 1903 g, which is 7.3% ($P \geq 0{,}001$) higher than the control and 2.0% higher than in 4th experimental group. The live weight of broilers in the 3rd and 4th experimental groups at the age of 35 days is 5.2-6.9% higher than in control group ($P \geq 0.01$). The absolute increase (Diagram 2) over the course of 35 days in the 2nd and 3rd test groups is almost the same - 1869-1855g and 123-137g by 7.1-7.9% ($P \geq 0.01$) but greater than in control group.

Fig:2 Absolute increase of broiler weight in 0-35 days. The absolute increase in the experimental groups was the highest in the 2nd experimental group and amounted to 1869 g, while in the 3rd and 4th experimental groups this figure was 1824-1855 g. During the growth period, the data of all experimental groups were higher than the control group

In the same groups, the highest daily gain was 53.0-53.2 g, which is 3.5-3.7 g higher than in control group.

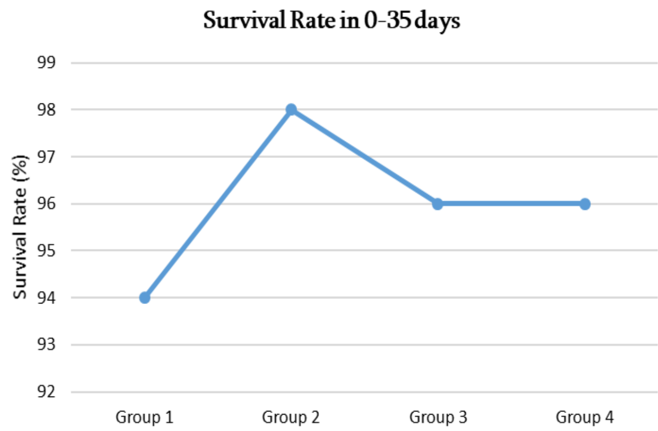

Fig.3 Broiler survival rate

Fig.3. Broiler survival rate in control group was 94%, which is 2-4% lower than in the test groups. The reason for the bird loss in the control group, even though they were given an antibiotic during the first period of growth, was a gastrointestinal disorder. In the first period of growth in the test groups, there was no bird loss due to gastrointestinal disorders. The main reason for the decline in these groups in late period of growth was myocardia.

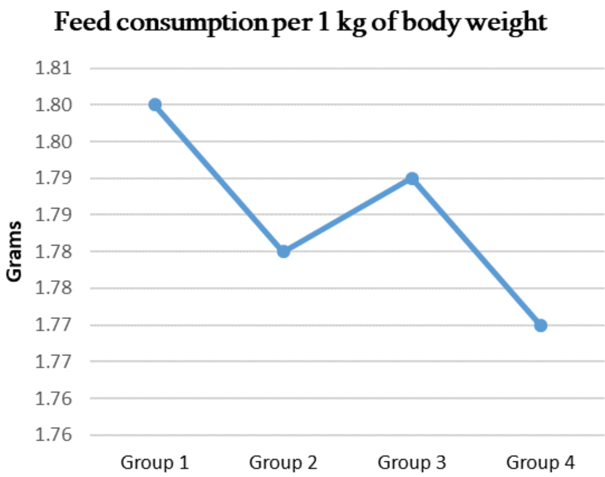

Fig:4 Feed consumption per 1 kg of body weight

Fig.4. Feed consumption per 1 kg body weight in the four groups is practically the same in the range of 1.77-1.80 kg, although the control group has a tendency to increase feed consumption.

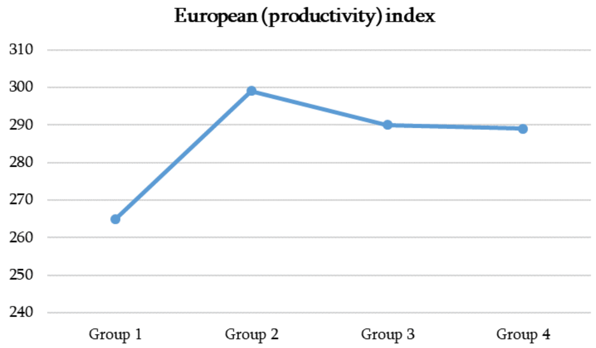

Fig.5 European (productivity) index

Fig.5 The productivity index in the experimental groups is almost the same and is quite high 289-299 units, which is 24-34 units higher than in control group.

At the age of 35 days was implemented slaughtering stage. Productivity was 81.1-81.6% in the control groups and 80.2% in the control groups, which is 1.4% less than in the test groups.

The study of the broiler category showed that 71% of the broiler body of the control group were in the first category, while this figure was 74.5% in the 2nd experimental group and 72.6% in the 3rd and 4th experimental groups. Non-standard meat was relatively high in the control and 4th experimental groups.

In order to study the effect of probiotics of new B. Subtilis on the chemical composition of broiler meat, 12-12 birds (6 females and 6 males) from each group were slaughtered at age of 35 days. From each bird from different parts of the body was taken 200 gr of sample meat, the meat was grounded and mixed and 400 gr of it was taken for analysis.

Chemical analysis of meat was performed in the testing laboratory of "Expertise +" Ltd. The results of the analysis showed that the water content varies between 75.96-78.49%, in the natural state, the protein content in the meat is practically the same in all four groups and varies between 17.2-17.9%. The fat content was highest at 5.48% in the control group and 3.3% in the lowest in the 4th experimental group. As for ash it was the same in all groups and ranged from 1.01-1.28%.

According to the research method, at the end of growth (35 days) we performed a general and biochemical analysis of broiler blood, which was conducted at the New Veterinary Clinic Ltd. And erythrocytes by 4.5-9%. These rates were highest in the second experimental group. As for leukocytes, this rate was almost the same in all groups and corresponds to physiological norms (21-22 109/l), also almost the same Color index, neutrophils, basophils, monocytes, lymphocytes, and erythrocyte sedimentation rate. These indicators correspond to physiological norms.

Blood biochemical analysis was performed. The content of total protein in blood serum, which plays a crucial role in the metabolism of carbohydrates and fats in the body, in the blood of the control group was slightly lower than the physiological norm and was 40.5 g / l, while in the experimental groups was within the norm and was 47-50 g / l. As for transferases, which are indicators of liver function within all three transferases within the norm and were almost the same in all groups.

Bacillus amyloliquefaciens application scheme is as follows

Group	Feed	Probiotic/Antibiotics	Quantity
Stage I			
I Group (control)	Combined feed with Antibiotic	Enrafloxacillin	100
II Group	Combined feed with Probiotic	B. amyloliquefaciens 1012 spore/gr 0.05%	100
III Group	Combined feed with Probiotic	B. amyloliquefaciens 1012 spore/gr 0.04%	100
IV Group	Combined feed with Probiotic	B. amyloliquefaciens 1012 spore/gr 0.03%	100

The growth dynamics of the broiler during the experiment

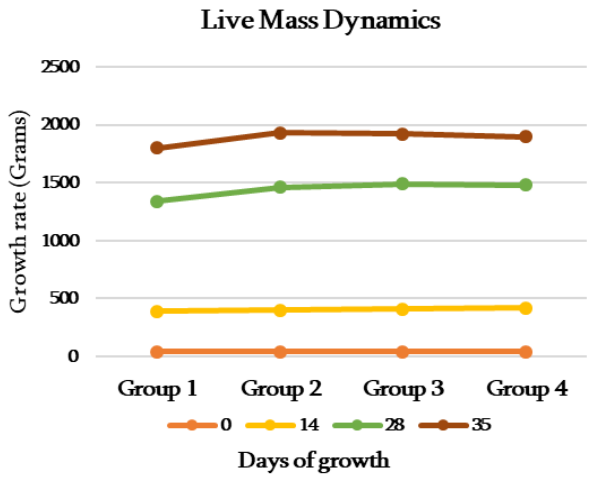

Fig.6. Live weight growth dynamic

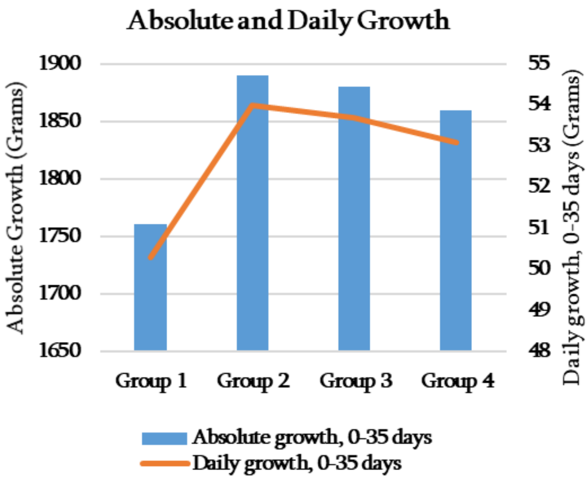

Fig.7. Daily and absolute weight gain in broiler

Fig. 6. The live weight of a one-day broiler is the same in all four groups of 39,7-40,2 g, which indicates a high uniformity of the test chickens. The live weight of the control broiler in forth group at age of 14 days was 420 g, Which is 7.7% higher compared to the control group ($P \geq 0{,}001$), as for the 2nd and 3rd experimental groups, they also exceeded the data of the control group by 2.4-5,0% in live weight.

The difference between the 2nd and 3rd experimental groups was 10-20 g compared to the 4th group. At 28 days of age, the live weight in the broiler of the experimental group was

1460-1490 g and exceeded the live weight of the broiler in the control group by 8.9-11.2% (P≥0,001). However at this age the relatively high live weight of the 2nd group broiler was 1490 g. And the difference in live weight between the experimental groups at this age is 0.0-1%, which is negligible. At the age of 35 days or at the end of the experiment, the highest live weight of group 2 broilers was 1930 g, which is 7.3% higher than the control (P≥0,001). As for the 3rd and 4th experimental groups, they were slightly 0.5-2.0% behind the 2nd group and exceeded the index of the control group by 5.5-6.7% (P≥0.01).

During the broiler rearing period (0-35 days) we studied the daily weight gain and absolute weight gain of the broiler.

Fig.7. The absolute weight gain of 1889.3 g was observed in the 2nd experimental group, while the lowest was 1760.2 g in the control group. Calculation according to the daily weight gain in groups showed that the broiler of all three experimental groups had the highest daily increments of 35.1-54.0 g during the 35-day period, while the lowest control group had 50.29 g. It should be noted, however, that the highest daily weight gain during the growing period was in the 2nd experimental group broiler at 54.0 g.

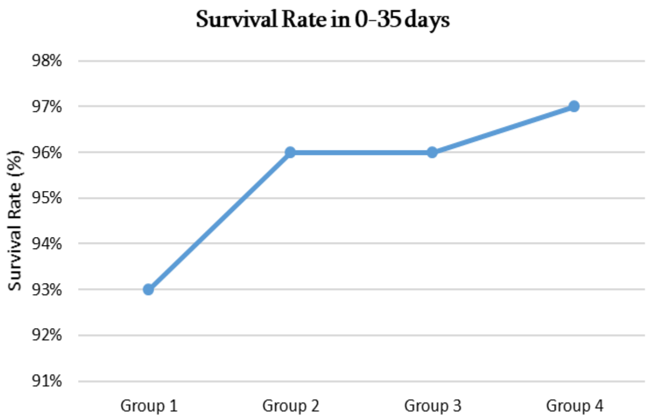

Fig.8 Broiler survival rate

Fig.8 Broiler survival rate by groups (0-35 days). Broiler survival is different in the experimental and control groups. The highest maintenance was observed in the 4th trial group-97%. The maintenance of the broiler of the 2nd and 3rd experimental groups was the same and amounted to 96%. The maintenance of the control group broiler was -93%, 3-4% less than in the experimental groups.

Feed consumption during the broiler rearing period is shown on the diagram.

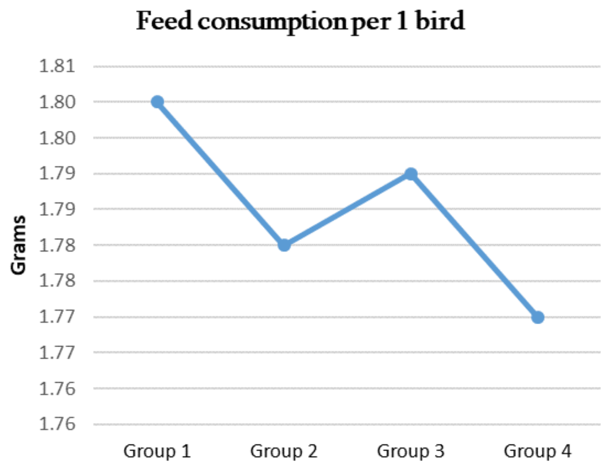

Fig.9 Feed consumption, 1 bird

Fig.9 Feed consumption per 1 bird ranged 3.5-3.6 kg in the experimental groups and in the control group it was 3.3 kg.

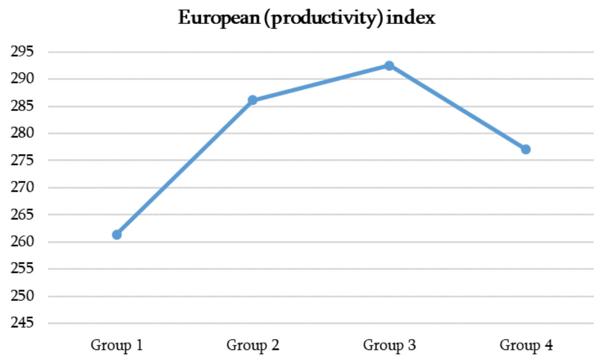

Fig.10. Broiler productivity index

Fig.10. The calculation of the productivity index showed that this indicator was the highest in the 3rd group - 292.5 units, which is 31.1 units higher than the control group. According to the mentioned parameter, the control group was 16-25 units behind the data of the second and fourth test groups.

The experiment showed that the optimal dose of new spore-producing Bacillus amyloliquefaciens probiotics in the first period (start, grower) of broiler rearing is 0.04-0.03%, and in the last period (finish) -0.05%

The application of new probiotics at a dose of 0.04% Bacillus subtilis and 0.05% dose of Bacillus amyloliquefaciens had a positive effect on broiler productivity

According to the data, the control group was 16-25 units behind of the second and fourth test groups. Thus, the experiment showed that the optimal dose of new spore-producing Bacillus amyloliquefaciens probiotics in the first period (starter, grower) of broiler rearing is 0.04-0.03%, and in the last period (finish) 0.05%.

At the end of the experiment, at the age of 35 days, we performed a control slaughter of the broiler, determined the slaughter solution and feed categories, the chemical composition of the meat, and some morphological and biochemical parameters of the blood. Slaughter yield was 81.7-82.1% in the second and third experimental groups, which is 1.3-1.7% higher than in the control.

The study of the bird body showed that 75% in the second and third experimental groups were in the first category, while in the control group the first category was 71%.

To study the chemical composition of the meat, samples were delivered to an accredited testing laboratory „Expertise + Ltd". Chemical analysis of meat showed that the water content in all groups is almost the same 73.17-76.76% also the ash content is almost the same 1.17-1.39%. The mass fraction of fat was highest in the fourth group and 6.57-7.49% in the control group, while the protein content was highest in the second and third groups of 21.77-22.32%. Some morphological and biochemical parameters of the blood were studied in the new veterinary clinic. Analyzes showed that the hemoglobin content in the blood in the second and third experimental groups was 129.5 g /l, which is 4.8-6.5% higher than in the control and fourth experimental groups.

As for the other indicators, they are almost the same in all groups and are within the norm. Similarly the biochemical parameters of the blood are almost the same in all four groups and are within the norm.

IV. Conclusion

Experiments conducted under production conditions to study the efficacy of the new probiotic *Bacilllus subtilis* allow us to draw the following conclusions:

1. Applicaion of Bacillus Subtilis in broiler breeding - using different doses of new probiotics - Live weight of broiler of experimental groups increased by 4.0-14.3% compared to broiler live weight of control group at 28 days of age.
2. At the last stage of broiler rearing at the age of 35 days, the highest live weight was observed in the 2nd experimental group broiler - 1903 g, which is 7.3% higher compared to the live group broiler live weight.
3. absolute gain at the last stage of broiler rearing at 35 days of age in the 2nd and 3rd experimental groups is almost the same at 1869-1855 g and 123-137 grams or 7.1-7.9% more than the control group.
4. During the experiment, broiler retention in the control group was 94%, which is 2-4% lower than in the experimental groups. The highest rate was observed in the 2nd experimental group where broiler retention was 98%.
5. Slaughter results showed that the outcome was 1.4% lower in the control group than in the experimental groups.
6. Overall biochemical analysis of broiler blood showed no significant changes between groups, although lower rates were observed in the control group than in the experimental groups.
7. The productivity index in the experimental groups is almost the same and is quite high 289-299 units, which is 24-34 units higher than in control group
8. The optimal dose of the new probiotic as a feed additive in the broiler feed is 0.04% of the new probiotic Bacillus Subtilis, produced on local agro-industrial raw materials.

Experiments conducted under production conditions to study the efficacy of the new probiotic *Bacilllus amyloliquefaciens* allow us to draw the following conclusions:

1. At the end of the start period and at the beginning of the grower (14 days), the highest live weight of the 4th group broiler was 420 g, which was 7.7% higher than the data of the control group, using different doses of the new probiotic Bacillus amyloliquefaciens. For the 2nd and 3rd experimental groups, they were 2. 4-5.0% higher than in control group.2. At 28 days of age, the live weight of the broiler of the experimental groups was 1460-1490 g, this figure was 8.9-11.9% higher than in control group.3. During the growing period (0-35 days) the highest rate of absolute increase was observed in 1889.3 g in the 2nd experimental group, and the lowest in the control group of 1760.2 g.
4. According to the daily growth rate, during the 35 days of growth, the highest result was observed in the experimental groups 53.1-54.0 g, in the control group this parameter was 50.3 g.
5. During the growing period of 35 days, the highest survive rate was observed in the broiler of the 4th experimental group, 97%, which is 4% higher than the control group (93%).
6. Feed conversion per 1 kg body weight in the experimental groups was 1.80-1.90 kg, and in the control group -1.83 kg
7. The productivity index is the highest in the 3rd experimental group - 292.5 units, which is 31 units higher than in control group
8. Slaughter results showed that the yield of meat in the experimental groups was 1.3-1.7% higher than that of the control group, while that of category I meat was 4% higher.
9. The optimal dose of a new probiotic spore forming Bacillus amyloliquefaciens produced on local agro-industrial raw materials, as a feed additive in the first period of growth of broiler (start, grower) is 0.03-0.04%, and in the last period of growth (finish) -0.05%.
10. The application of new spore forming probiotics at a dose of 0.04% Bacillus subtilis and 0.05% dose of Bacillus amyloliquefaciens had a positive effect on broiler productivity.

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References

[1] European Commission. Ban on Antibiotics as Growth Promoters in Animal Feed Enters into Effect; Press Release Database. European Commision: Brussels, Belgium, 2005. Available online: http://europa.eu/rapid/pressrelease_IP-05-1687_en.htm (accessed on 2 October 2020).

[2] Probiotics (Direct-Fed Microbials) in Poultry Nutrition and Their Effects on Nutrient Utilization, Growth and Laying Performance, and Gut Health: A Systematic Review. Jha R, Das R, Oak S, Mishra P.Animals (Basel). 2020 Oct 13;10(10):1863. doi: 10.3390/ani10101863.

[3] Advantages of Bacillus-based probiotics in poultry production, Author links open overlay

panelU.RamluckenabcR.LallooaY.RoetsaG.MoonsamyaC. Jansenvan RensburgbM.S.Thantsha, Received 13 January 2020, Revised 16 July 2020, Accepted 14 August 2020, Available online 15 August 2020.

[4] Smialek, M.; Burchardt, S.; Koncicki, A. The influence of probiotic supplementation in broiler chickens on population and carcass contamination with Campylobacter spp-Field study. Res. Vet. Sci. 2018, 118, 312–316.

[5] Wang A, Ran C, Wang Y, Zhang Z, Ding Q, Yang Y, Olsen RE, Ringø E, Bindelle J, Zhou Z (2019) Use of probiotics in aquaculture of China-a review of the past decade. Fish Shellfish Immunol 86:734–755. https://doi.org/10.1016/j.fsi.2018.12.026

[6] Bacillus probiotics: an alternative to antibiotics for livestock production, S. Mingmongkolchai, W. Panbangred, First published: 09 January 2018, https://doi.org/10.1111/jam.13690.

[7] Elisashvili V., Berikashvili V., Sokhadze K., Kachlishvili E., Chikindas M.L. *Bacillus amyloliquefaciens* Spore Production Under Solid-State Fermentation of Lignocellulosic Residues. Probiotics and Antimicrobial Proteins. 2018, 10, 755–761

[8] Gadde, U.D.; Kim, W.H.; Oh, S.T.; Lillehoj, H.S. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. Anim. Health Res. Rev. 2017, 18, 26–45.

[9] Khardziani T., Kachlishvili E., Sokhadze K., Elisashvili V., Weeks R., Chikindas M.L., Chistyakov V. Elucidation of *Bacillus subtilis* KATMIRA 1933 potential for spore production in submerged fermentation of plant raw materials. Probiotics and Antimicrobial Proteins. 2017, 9, 435-443.

[10] Khardziani T., Sokhadze K., Kachlishvili E., Chistyakov V., Elisashvili V. Optimization of enhanced probiotic spores production in submerged cultivation of *Bacillus amyloliquefaciens* B-1895. J. Microbiol Biotechnol Food Sci. 2017, 7, 132-136

[11] Bajagai, Y.S.; Klieve, A.V.; Dart, P.J.; Bryden, W.L. Probiotics in Animal Nutrition–Production, Impact and Regulation by FAO Animal Production and Health Paper No. 179; Harinder, P.S., Ed.; FAO: Rome, Italy, 2016; ISBN 978-92-5-109333-7.

[12] Abd El-Tawab, M.M.; Youssef, I.M.; Bakr, H.A.; Fthenakis, G.C.; Giadinis, N.D. Role of probiotics in nutrition and health of small ruminants. Pol. J. Vet. Sci. 2016, 19, 893–906.

[13] Ammar Algburi, Carla Cugini, Emily Walsh, Anna Volski, Safety Properties and Probiotic Potential of Bacillus subtilis KATMIRA1933 and Bacillus amyloliquefaciens B-1895, Advances in Microbiology 2016, pp 432-452

[14] Aga, D. S., Lenczewski, M., Snow, D., Muurinen, J., Sallach, J. B., and Wallace, J. S. (2016). Challenges in the measurement of antibiotics and in evaluating their impacts in agroecosystems: a critical review. J. Environ. Qual. 45, 407–419. doi: 10.2134/jeq2015.07.0393.

[15] Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O. Antibiotic resistance–The need for global solutions. Lancet Infectious Diseases. 2013;13:1057-1098.

[16] Guo, J.J., Liu, K.F., Cheng, S.H., Chang, C.I., Lay, J.J., Hsu, Y.O., Yang, J.Y. and Chen, T.I. (2009) Selection of probiotic bacteria for use in shrimp larviculture. Aquacult Res 40, 609–618

[17] Arun Panda, Sv Rama Rao, M V L N Raju, Effect of probiotic (Lactobacillus sporogenes) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders, Journal of the Science of Food and Agriculture 2008, pp.43 – 47

[18] K M Tuohy, M Pinart-Gilberga, M Jones, L Hoyles, A L McCartney, G R Gibson, Survivability of a probiotic Lactobacillus casei in the gastrointestinal tract of healthy human volunteers and its impact on the faecal microflora, J Appl Microbiol. 2007, pp.1026-1032

[19] Castanon, J.I., 2007. History of the use of antibiotic growth promoters in European poultry feeds. 493 Poultry Science 86, 2466-2471.

[20] T.M. Barbosa, C.R. Serra, R.M. La Ragione, M.J. Woodward, A.O. Henriques, Screening for bacillus isolates in the broiler gastrointestinal tract, Appl. Environ. Microbiol., 71 (2005), pp. 968-978

[21] V. Kurtoğlu , F. Kurtoğlu, B. Coşkun, Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol, Food Additives and Contaminants 2004, pp.817-823