



New probiotic produced from spore forming *Bacillus* cultivated on local agro-industrial wastes substituting antibiotic in broiler nutrition

A. Chkuaseli^a, A. Chagelishvili^a, M. Khutsishvili-Maisuradze^a, V. Elisashvili^a,
T. Khardziani^a, T. Lashkarashvili^b, G. Chagelishvili^a, R. Lapachi^a,
K. Samkurashvili^c, V. Berikashvili

^aAgricultural University of Georgia, Kakha Bendukidze University Campus; 240, David Aghmashenebeli Alley, Tbilisi, 0159, Georgia

^bLTD „Nutrimax“; L. Gogoberidze 67^b, Tbilisi, 0165, Georgia

^cLTD „Roster“; Gardabani, Teleti, 1300, Georgia

Received: 02 July 2021; Accepted: 25 July 2021

ABSTRACT

In Georgia, poultry farms continue to use antibiotics as a food supplement to prevent poultry diseases. Given these circumstances and the benefits of using probiotics, local market requirements, availability of resources and the lack of probiotics production in the South Caucasus, the Agricultural University of Georgia team, with the financial support of the “Shota Rustaveli National Science Foundation of Georgia”, has conducted research which enabled us to study the physiological mechanisms that regulate and improve the use of *Bacillus amyloliquefaciens* spore forming probiotics in broiler nutrition. The growing demand to increase and improve the quality of poultry production has put on the agenda the search for alternative methods of removing the antibiotic load in the bird's body. To achieve this, new strains of *Bacillus amyloliquefaciens* were updated in the collection, which were tested in broiler feed. A new probiotic from spore forming *Bacillus amyloliquefaciens* cultivated on local agro-industrial raw materials was mixed with 0.05%, 0.04% and 0.03% of the feed in the broiler plant as a food supplement in the form of a feed additive in 3 test groups. In control group was applied feed with antibiotic. Both broiler and control group broilers were fed a complete combined feed in appropriate phases, which met the broiler's demand for nutrients, minerals, and biologically active substances. Based on the experiment, it was found that the optimal dose of the new probiotic *Bacillus amyloliquefaciens*, as a food additive in the first period of growth (start, grower) is 0.04-0.03%, and in the concluding period (finish) – 0.05%.

Keywords: Broiler, Antibiotic, Probiotic, Efficiency, Experiment, Probiotic optimal dose.

*Corresponding author: Amrosi Chkuaseli; E-Mail address: a.chkuaseli@agrundi.edu.ge

Urgency of the issue

Due to the growing demand for broiler meat, both the number of poultry farms and their capacity have increased significantly. The use of antibiotics in the production of broiler meat is accepted as a prophylactic as well as the use of growth-promoting [1] antibiotics. The use of antibiotics led not only to the emergence of pathogens resistant to multibiotic drugs but also to a reduction in the microflora

and immune function [2] required in the broiler, resulting in production losses and a significant increase [3] in overall costs. Potential transfers of antibiotic resistance from animals to humans [4] have been introduced. Due to this problem, the use of antibiotics as growth stimulants has been banned in Europe and other developed countries [5]. It has therefore become mandatory to replace antibiotics with other effective means. The use of probiotics as micro-organisms that create a natural

protective barrier between the broiler organism and pathogens has become the most realistic natural alternative to gastrointestinal antibiotic therapy [6], which promotes broiler productivity and product safety [7].

The *Bacillus subtilis* and *Bacillus amyloliquefaciens* application in broiler feed are characterized by high adaptation to environmental conditions and high growth rate on plant raw materials. Thermoresistant spores are stable to long-term storage without refrigeration, moreover, the spores have the ability to survive in the gastrointestinal tract even at low pH levels [8-10]. Thus, the full dose of spore bacteria invariably reaches the small intestine, which is not the case for all species of *LactoBacillus* [11,12]. 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, the vegetative forms of *Bacillus* and *Bacillus amyloliquefaciens* produce extracellular enzymes (protease, cellulase, xylanase, pectanase and lipase) that promote the absorption of nutrients [13]. The use of probiotics in broiler production has made it possible to improve: broiler maintenance, live weight, feed conversion [14] economic efficiency [15-17].

Aim and objective of research

Probiotics produced on agro-industrial waste raised the issue of studying the effectiveness of the use of new probiotics from *Bacillus subtilis* and *Bacillus amyloliquefaciens* as a dietary supplement of antibiotic in broiler feeding, which formed the basis of the research work.

The aim of the study was to determine the optimal dose of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in broiler feeding.

Testing of probiotic drugs, confirmation of its best effect was carried out in the poultry enterprise "Roster" Ltd. The biological efficacy, feed conversion, broiler growth and productivity of the new probiotic drug *Bacillus subtilis* and *Bacillus amyloliquefaciens* were evaluated. The study was conducted on several experimental and control groups.

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 [18]. 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. [19-21]. 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 was fed phased whole food, the zootechnical analysis were carried out in the accredited laboratory "Chirina" Ltd. For the experimental groups broiler, probiotics of *Bacillus Subtilis* and *Bacillus Amyloliquefaciens* were mixed separately with feed at the required concentration using a rotary mixer.

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. *Bacillus subtilis* test scheme

Group	Feed	Probiotic/Antibiotics	Quantity
Stage I			
I Group (control)	Combined feed with probiotic	Enrafloxacillin	100
II Group	Combined feed with probiotic	<i>B. subtilis</i> 10 ⁸ spore/gr 0.05%	100
III Group	Combined feed with probiotic	<i>B. subtilis</i> 10 ⁷ spore/gr 0.04%	100
IV Group	Combined feed with probiotic	<i>B. subtilis</i> 10 ⁶ spore/gr 0.03%	100

According to the experimental scheme, the first group was the control group, during the whole period of growth (1-35 days) they received a complete combined feed with no probiotics and in the first days of growth (1-3 days) they took the antibiotic Enrafloxacillin with water. The second, third, and

fourth groups were experimental and consumed the probiotic *Bacillus subtilis* in the amount of 0.05%, 0.04%, and 0.03%, respectively.

The growth dynamics of the broiler during the experiment.

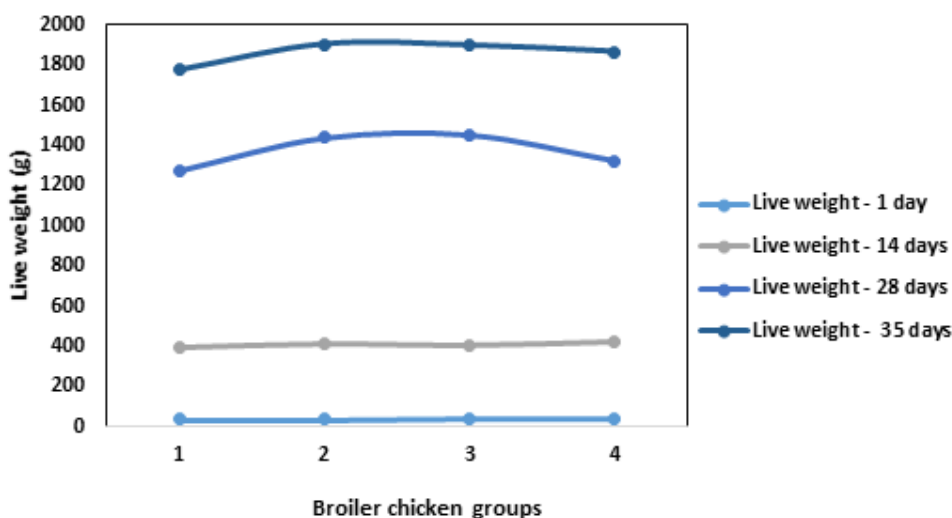


Fig. 1. Live weight growth dynamic

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.

During the experiment we also studied the daily weight gain of the broiler.

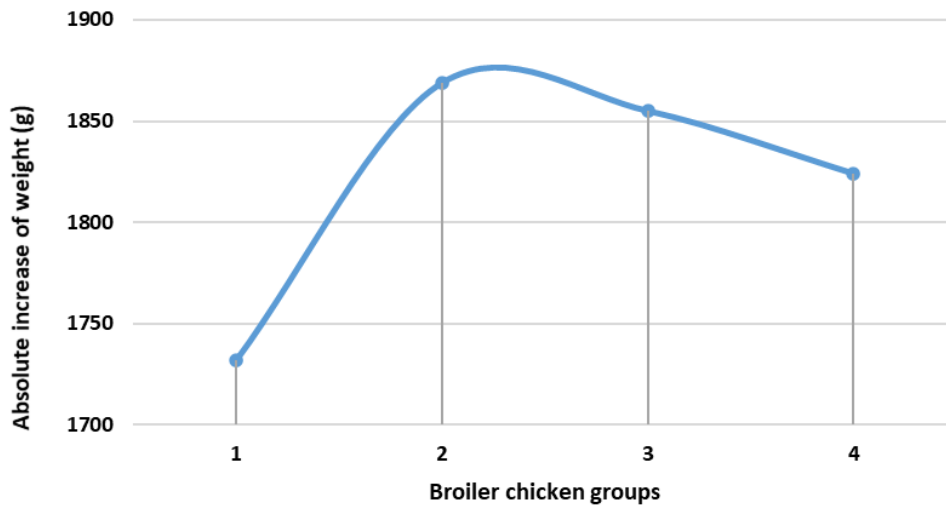


Fig. 2. Broiler daily weight gain

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.

During the experiment was studied an absolute increase of weight in groups.

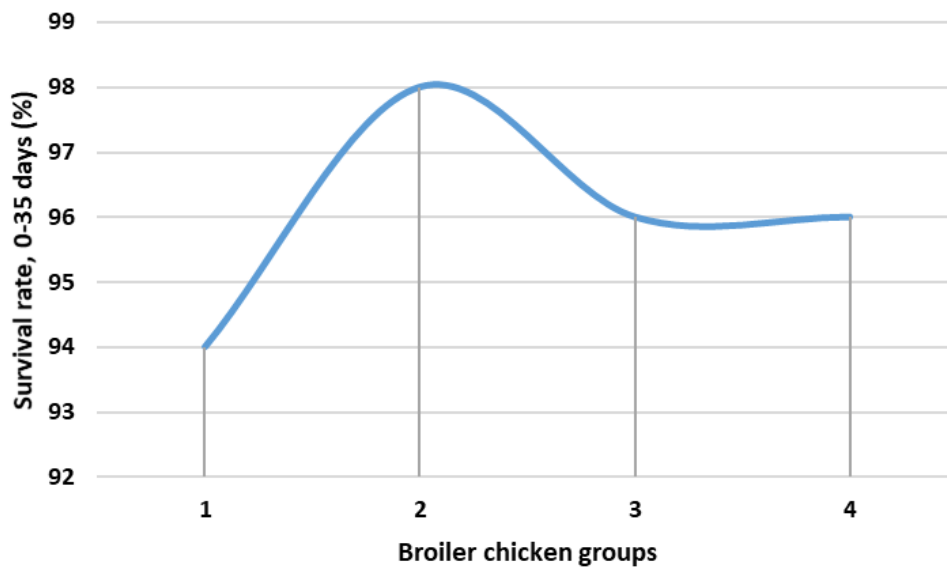


Fig. 3. Absolute increase of weight of broilers

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.

Broiler survival rate during the age of 35 days period was different in groups.

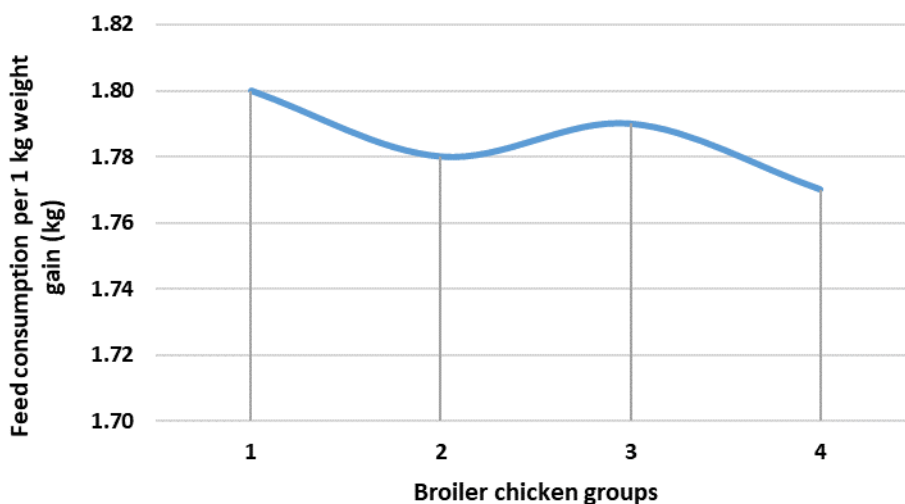


Fig. 4. Broiler survival rate

Broiler survival rate in control group was 94%, which is 2-4% lower than in the test groups (Diagram 4). 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.

During the broiler rearing period was studied the feed consumption per 1 kg of body weight.

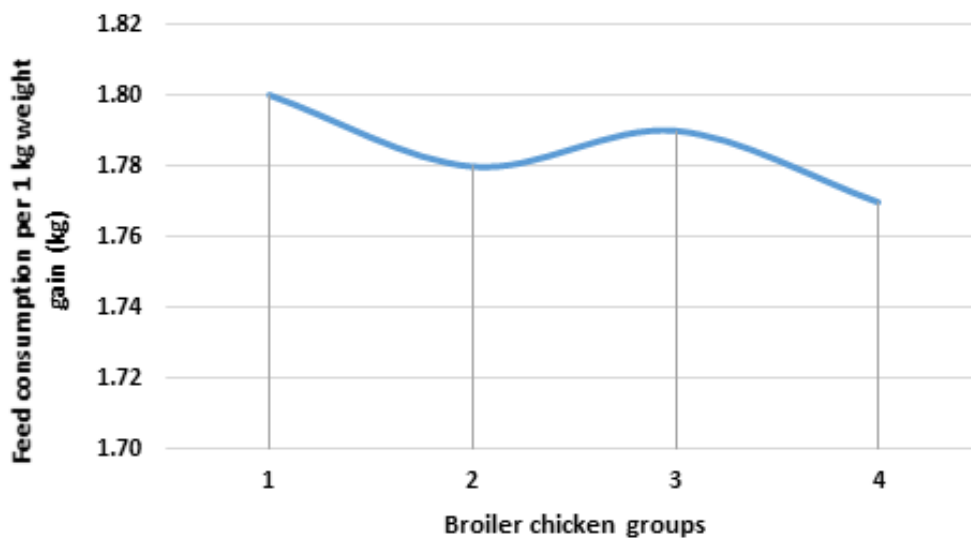


Fig. 5. Feed consumption per 1 kg of body weight

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.

At the end of the experiment we calculated the European (productivity) index of the broiler.

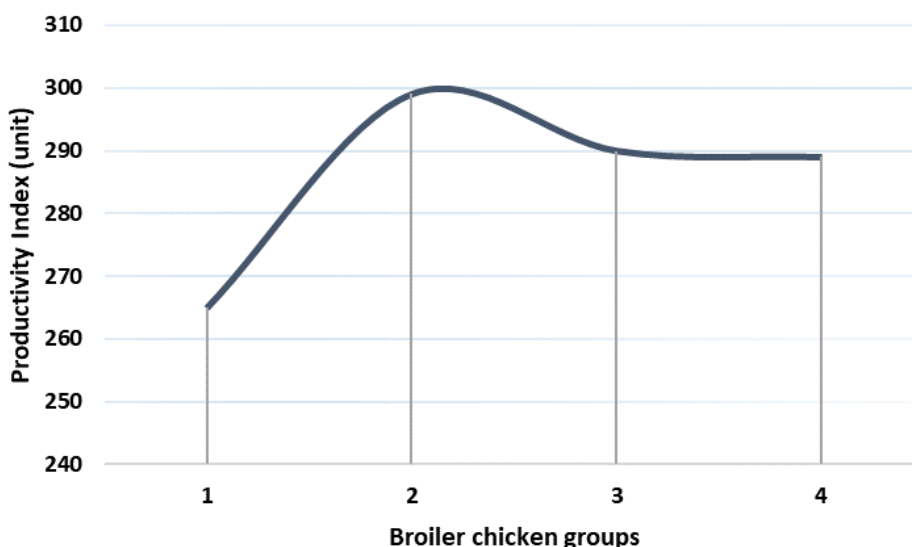


Fig. 6. European (productivity) index

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.

Thus, the optimal dose of the new probiotic as a feed additive in the broiler feed is 0.04% of the new is as follows:

probiotic *Bacillus Subtilis*, produced on local agro-industrial raw materials.

In addition to *Bacillus Subtilis*, our goal was to use the new spore-producing *Bacillus Amyloliquefaciens* and determine its optimal dose.

Bacillus amyloliquefaciens application scheme

Table 2. *Bacillus amyloliquefaciens* test scheme

Group	Feed	Probiotic/Antibiotics	Quantity
Stage II			
I Group (control)	Combined feed with probiotic	Ernafloracillin	100
II Group	Combined feed with probiotic	<i>B. amyloliquefaciens</i> 10 ⁸ spore/gr 0.05%	100
III Group	Combined feed with probiotic	<i>B. amyloliquefaciens</i> 10 ⁷ spore/gr 0.04%	100
IV Group	Combined feed with probiotic	<i>B. amyloliquefaciens</i> 10 ⁶ spore/gr 0.03%	100

According to the experimental scheme, the first group was the control group, during the whole period of growth (1-35 days) they received a complete combined feed with no probiotics and in the first days of growth (1-3 days) they took the antibiotic Ernafloracillin with water. The second, third, and

fourth groups were experimental and consumed the probiotic *Bacillus amyloliquefaciens* in the amount of 0.05%, 0.04%, and 0.03%, respectively.

The growth dynamics of the broiler during the experiment.

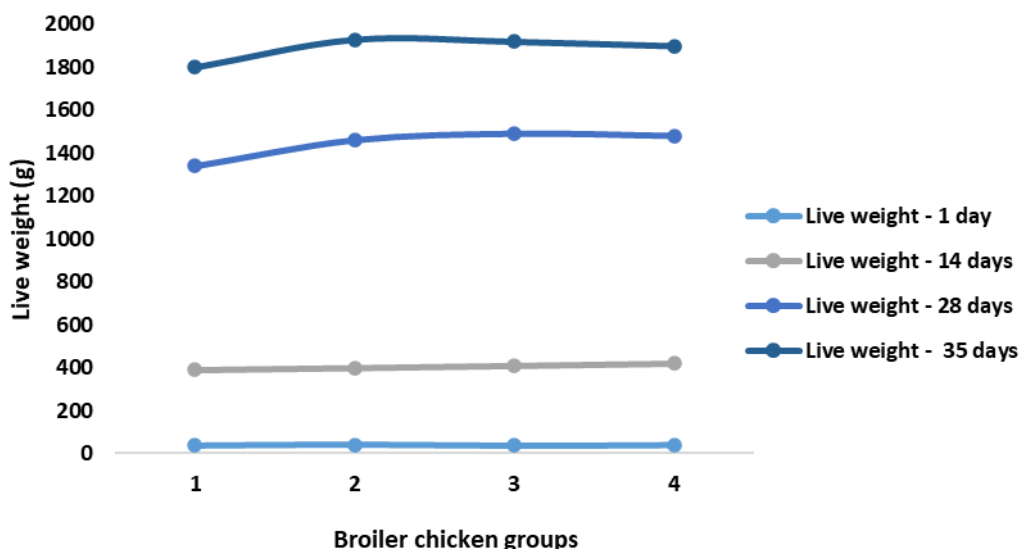


Fig. 7. Broiler live weight growth dynamic

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 fourth 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% ($P \geq 0,001$). 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 ($P \geq 0,001$). 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% ($P \geq 0,01$).

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

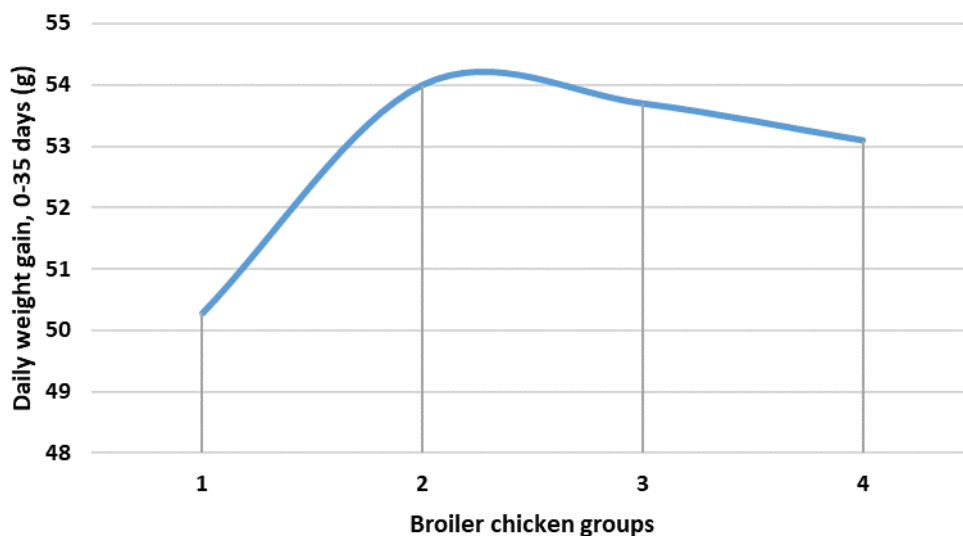


Fig. 8. Daily weight gain of the broiler

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.

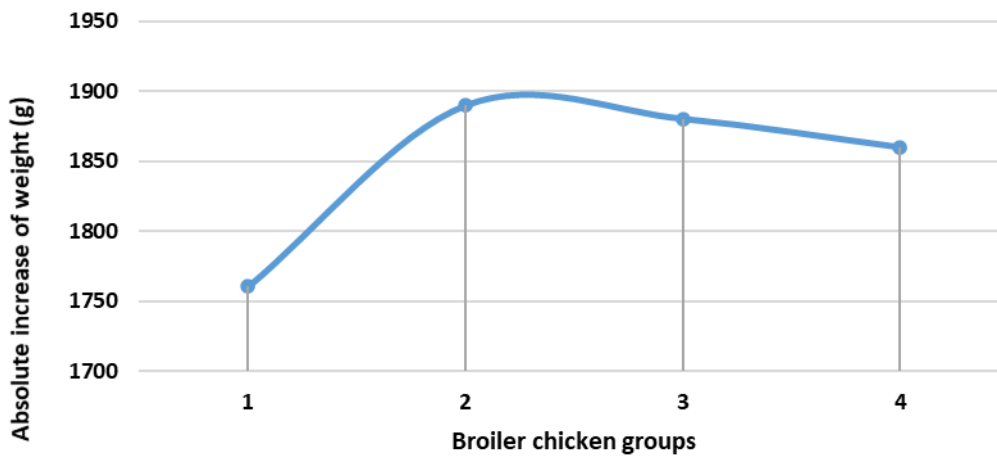


Fig. 9. Absolute increase of weight

Absolute increase of broiler 0-35 days. The absolute increase in the experimental groups was the highest in the 2nd experimental group and amounted to 1890 g, while in the 3rd and 4th experimental groups this figure was 1880-1860 g. During the

growth period, the data of all experimental groups are higher than those of the control group.

As for the survival rate of the broiler during the growing period, the data in the experimental and control groups are shown in the diagram.

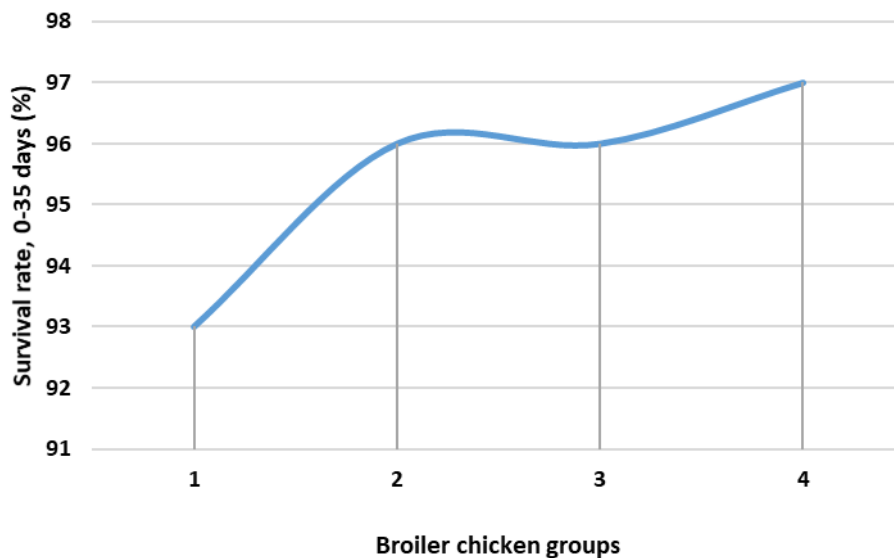


Fig. 10. Broiler survival rate

Broilers 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.

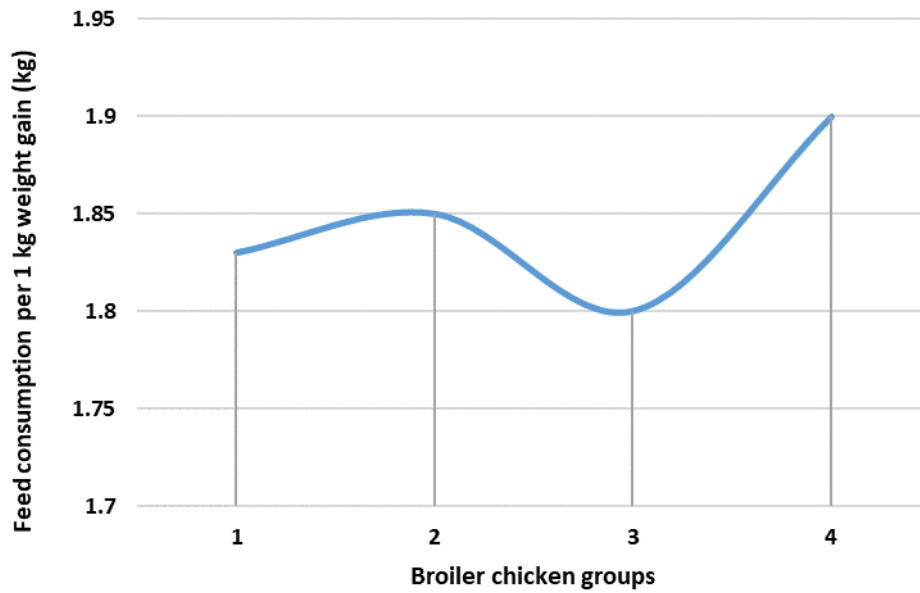


Fig. 11. Feed consumption per 1 kg of body weight

Feed consumption per 1 kg of body ranged from 1.85 to 1.90 kg in the experimental groups and in

the control group it was 1.83 kg.

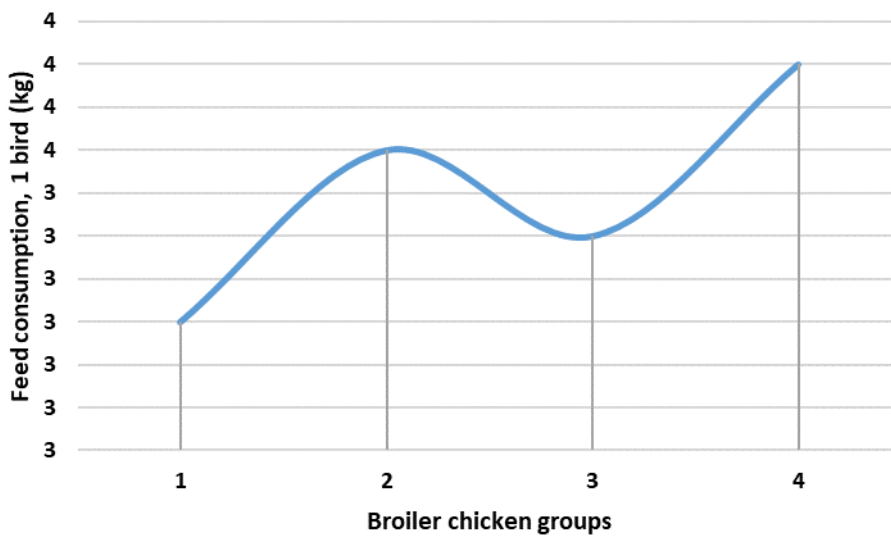


Fig. 12. Feed consumption, 1 bird

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.

At the end of the experiment we calculated the European (productivity) index of the broiler.

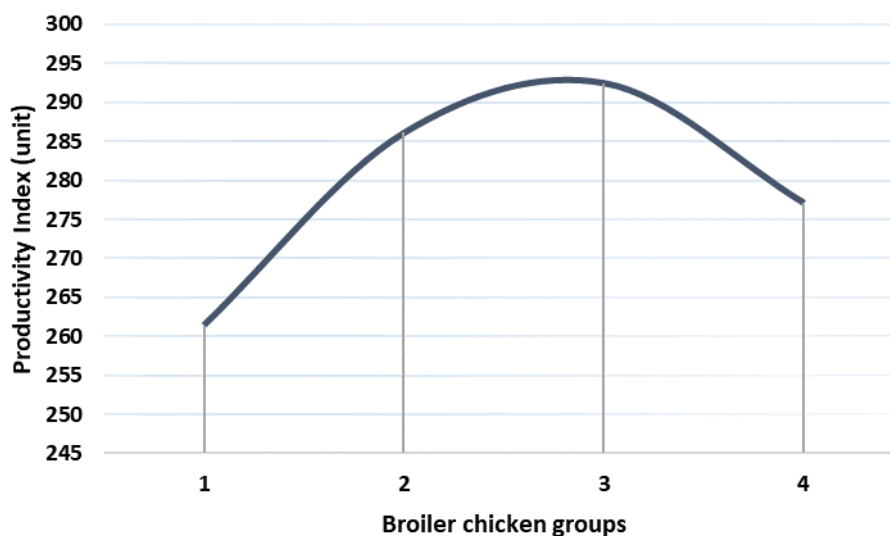


Fig.13. Broiler productivity index

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.

Acknowledgement

The research was conducted with the financial assistance of “Shota Rustaveli National Science Foundation of Georgia” (Grant Project CARYS-19-2268).

References

- [1] World Health Organization. Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment; World Health Organization: Geneva, Switzerland (2003); No. WHO/CDS/CPE/ZFK/2004.7.
- [2] Roth, N.; Käsbohrer, A.; Mayrhofer, S.; Zitz, U.; Hofacre, C.; Domig, K.J., The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview, *J. Poult. Sci.*98 (2019) 1791–1804.
- [3] A. Sundsfjord, M. Sunde. *Tidsskr Nor Laegeforen*, Antimicrobial resistance after antibiotic use in animals- impact on human health, 98 (2008) 2457-61.
- [4] M.T. Madigan, J.M. Martinko, K.S. Bender, F.H. Buckley, D.A. Stahl, *Brock Biology of Microorganisms*. 14th ed. Illinois, J. Pearson International (2014) 1006.
- [5] U. Gadde, W.H. Kim, S.T. Oh, H.S. Lillehoj, Alternatives to antibiotics for maximizing growth performance and feed efficiency in

- poultry: a review, *J. Animal Health Res Rev* (2017)26-45.
- [6] A.Grant, G.Gay, H.S. Lillehoj, [Bacillus spp. as](#) direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry, *J. Avian Pathol.* 47(4)(2018) 339-351.
- [7] ERL. Pelicano, P.A. de Souza, HBA. de Souza, Oba A, EA. Norkus, LM. Kodawara, T.M.A. de Lima, Effect of different probiotics on broiler carcass and meat quality, *J. Poult. Sci.*5 (2003) 207–214.
- [8] Hong, H.A., Duc, L.H. and Cutting, The use of bacterial spore formers as probiotics, *J. FEMS Microbiol Rev.*29 (2005) 813–835.
- [9] M.Brashears , A.Amezquita, D.Jaroni, Lactic Acid Bacteria and Their Uses in Animal Feeding to Improve Food Safety, *J. Advances in Food and Nutrition Research* (2005).
- [10] 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**, *J. Appl. Environ. Microbiol.*71 (2005) 968-978.
- [11] J.I. Cañanón, History of the use of antibiotic growth promoters in European poultry feeds, *J. Poultry Science.*86 (2007) 2466-2471.
- [12] [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) 1026-1032.
- [13] 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., Selection of probiotic bacteria for use in shrimp larviculture, *J. Aquacult Res* 40 (2009) 609–618.
- [14] Chaucheyras-Durand, F. and Durand, H., Probiotics in animal nutrition and health, *J. Benef Microbes* (2010) 1, 3–9.
- [15] [V. Kurtoğlu](#) , [F. Kurtoğlu](#), [B. Coşkun](#), Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol, *J. Food Additives and Contaminants* (2004) 817-823.
- [16] Duc, L.H., Hong, H.A., Barbosa, T.M., Henriques, A.O. and Cutting, Characterization of *Bacillus* probiotics available for human use, *J. Appl Environ Microbiol.*70 (2004) 2161–2171.
- [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, *J. Science of Food and Agriculture* (2008) 43–47.
- [18] [Algburi](#), [C. Cugini](#), [E.Walsh](#), [A. Volski](#), Safety Properties and Probiotic Potential of *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895, *J. Advances in Microbiology* (2016) 432-452.
- [19] T. Khardziani, E. Kachlishvili, K. Sokhadze, V. Elisashvili, R. Weeks, M.L. Chikindas, V. Chistyakov, Elucidation of *Bacillus subtilis* KATMIRA 1933 potential for spore production in submerged fermentation of plant raw materials, *J. Probiotics and Antimicrobial Proteins.*9 (2017) 435-443.
- [20] T. Khardziani, K. Sokhadze, E. Kachlishvili, V. Chistyakov, V. Elisashvili, Optimization of enhanced probiotic spores production in submerged cultivation of *Bacillus amyloliquefaciens* B-1895, *J. Microbiol Biotechnol Food Sci.* 7 (2017) 132-136.
- [21] V. Elisashvili, M.khutsishvili, A.Chagelishvili et al., -Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation, *J. Bioscience of Microbiota, Food and Health.*34 (2015) 25-28.