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(Research Article)

Medicinal mushrooms and Bacillus probiotics as an alternative to antibiotics and their effect on broiler productivity

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Abstract

Antibiotics as feed additives still have been used in Georgian poultry farms with the purpose of disease prevention and growth stimulation. Taking global trends and local resources into account, this research has set a goal of elaboration of alternative means to antibiotics with the use of mycelial biomass of medicinal mushrooms. Under laboratory conditions, we have obtained mycelial biomass of *Ganoderma lucidum* and *Pleurotus ostreatus* on the local agro-industrial substrates. The obtained biomass after drying and milling has been added to broiler feed as a functional additive. In the course of the fourth and final trial conducted at the table (meat-type) poultry broiler production, probiotics *Bacillus* have been also added in addition to medicinal mushrooms (*Ganoderma lucidum* and *Pleurotus ostreatus*) in a feed ration as a feed additive.

Broilers' live weight gain, feed conversion rate (FCR), survival rate and common health parameters have been assessed during all four trials.

The results showed that application of medicinal mushrooms and probiotics has had a beneficial effect on broilers' growth and productivity indices. Based on the carried-out tests, it can be said that the elaborated additive is a safe and effective alternative to antibiotics.

Keywords: Broiler; Nutrition; Feed; Antibiotic; Medicinal Mushrooms; Probiotics; Experiment

1. Introduction

Poultry meat is one of the most accessible and widely used sources of animal protein, consumption of which on a global scale, including in Georgia, has been significantly increased over recent years [1]. According to 2020 data, a share of broiler meat in global production of total animal-derived meat has reached 40%, that emphasizes its importance both as a food product and from the viewpoint of economic resources [2].

In several counters, intensive production of broiler meat is closely related to wide use of antibiotics, both for disease prevention and growth stimulation purposes [3]. However, promiscuous and excessive application of antibiotics to a significant degree promoted development of antimicrobial resistance (AMR), which has a negative effect on both poultry and human health [4,5,6].

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There is an active search for alternative solutions aimed to replacement of antibiotics. Special attention is paid to compounds of natural origin (feed additives), including medicinal mushrooms and probiotic cultures with antibacterial, immunomodulatory and antioxidant properties [7, 8].

Based on this fact, the work deals with assessment of efficiency of medicinal mushrooms - *Ganoderma lucidum*, *Pleurotus ostreatus* and probiotic bacteria *Bacillus subtilis* and *Bacillus amyloliquefaciens*, produced on the basis of local agro-industrial waste. This study not only represents a scientific novelty, but also demonstrates practical opportunities, which will promote poultry productivity growth, feed consumption reduction and food safety improvement in the local poultry keeping sector.

2. Method

- Mycelial biomass of *Ganoderma lucidum* and *Pleurotus ostreatus* was produced under laboratory conditions using wheat bran, as the growth substrate. The mushrooms were cultivated in solid-state fermentation (SSF) systems; biomass was harvested, dried, and analyzed for basic biochemical and antibacterial properties [9].
- SSF was used to cultivate *Bacillus subtilis* IMB-73 and *Bacillus amyloliquefaciens* IMB-79 using wheat bran and cilantro as lignocellulosic substrates. The fermentation was carried out under optimized conditions to obtain high spore yields.[10].
- Weighing method – for assessment of weight gain and growth dynamics.
- Count method – for poultry mortality rate.
- Method for consumed feed calculation.

3. Research results under poultry /broiler factory conditions

3.1. Live weight dynamics

In zootechnics, observation over growth of agricultural animals and poultry is made in different age periods through their periodical weighing [11,12,13]. During our experiment, a live weight gain of broilers has been studied at 1, 7, 14, 28, 35-37-days age. Broilers' live weight dynamics is given in Table №1, while for greater clarity their values are presented in Diagrams 1, 2, 3 and 4.

Table 1 №1- Broilers' live weight dynamics

	I test				
	Age/Days				
	1	**7**	**14**	**28**	**37**
	M±m	M±m	M±m	M±m	M±m
Control	40.6±1.08	175.07±2.39	445.25±2.48	1350.57±10.54	1980.68 ± 18.93
I	39.95±1.12	179.16±2.92	460.26±3.94	1405.68±14.90	2010.98 ± 16.36
II	40.5±1.06	182.18±2.38	475.33±9.83	1430.30±23.26	2050.93 ± 35.47
	II Test				
	Age/Days				
	1	**7**	**14**	**28**	**37**
	M±m	M±m	M±m	M±m	M±m
Control	38.84±1.13	175.89±2.02	444.43 ±2.28	1295.88±11.45	1854.77±12.93
I	39±1.10	182.25±2.50	478.64 ±10.45	1353.13±18.97	1908.55±15.33
II	40.26±1.22	182±2.41	473.42 ±9.08	1346.32±20.37	1900.67±16.27
III	39.27±1.01	182.19±2.55	478.35 ±11.45	1356.04±18.58	1909.69±23.34
IV	41±1.28	181.95±2.39	470.88 ±9.74	1347.42±22.54	1897.36±14.55

	III Test				
	Age/Days				
	1	**7**	**14**	**28**	**37**
	M±m	M±m	M±m	M±m	M±m
Control	40.33±1.06	178.82±4.18	450.26±4.72	1362.91±23.54	1863.00±15.14
I	42.04±1.23	194.73±2.66	531.94±24.66	1497.27±32.85	1963.59±48.79
II	41.78±1.25	191.96±2.89	507.38±17.89	1415.31±11.42	1918.81±17.18
	IV Test				
	Age/Days				
	1	**7**	**14**	**28**	**37**
	M±m	M±m	M±m	M±m	M±m
Control	40.61±1.05	189.82±4.73	460.76±2.59	1374.09±23.62	1877.12±15.15
I	42.16±1.21	205.74±3.34	567.69±35.26	1549.25±54.11	2026.20±48.79
II	42.02±1.01	203.29±3.42	542.64±35.93	1524.79±40.26	2019.27±40.69

During all four tests technological parameters of broilers survival rate were identical for all groups and satisfied technological standards of broiler's breeding. As for feed ration, it was in compliance with nutritional standards of broilers cross „ROSS-308" from the viewpoint of their upbringing in phases. During all tests, 100 one-day broiler chickens from each group were exposed to tests. Broiler chickens of the control group were fed by wholesome compound feed (in bulk) and in the beginning, during 1st-5th days of their breeding they took medicinal preparation /antibiotic Enrofloxacin along with water. Over the course of three production tests, broilers of all test groups, in addition to main wholesome compound feed, took in different doses the medicinal mushrooms *Ganoderma Lucidum* 447 and *Pleurotus ostreatus* 2191 according to experimental design, while during the fourth production test, probiotics *Bacillus subtilis* and *Bacillus amyloliquefaciens* were added to broiler's main wholesome compound feed along with the above-mentioned medicinal mushrooms, also according to experimental design.

It is seen from analysis of Diagram №1 that during the first production test, a live weight of one-day broiler in all three groups (1 control and 2 test ones) was almost the same and varied between 39.95- 40.60 grams. On the 7th day of breeding a live weight of broilers of the control group was equal to 175.07 grams, while broiler weights of test groups - 179.16-182.18 grams, respectively, that is 2.3-4% higher compared to weight of broilers from the control group. ($P \geq 0.5$ compared to the third group). As for variation coefficient, broiler of the second test group was distinguished by equaled values of live weight and variation coefficient comprised 1.31%. On the 14th of upbringing a live weight of broilers from the control group comprised 445.25 grams, which is 3.3-6.7% lower compared to broilers of the test groups (460.26-477.33 grams; $P \geq 0.01$). As for variation coefficient, it was the lowest for the control group, and the highest – for the second test group. On the 28th day of breeding, broiler of the second test group was distinguished by the highest live weight 1430.30 grams, which is almost 80 grams higher compared to broiler of the control group. In the end of production, on the 37th day, a live weight of broilers from the test group varied between 2010.98-2050.93 grams, while for broilers of the control group it was equal to 1980.68 grams, which is almost 3.5% lower compared to broiler of the test groups. Regarding variation coefficient, similar to previous age periods, here the lowest index was determined for broilers of the control group, while the highest one – for broilers of the second test group. As for live weight gain dynamics for broilers of the test group, in all age periods a relatively high live weight was recorded for broilers of the second test group, where the medicinal mushroom *Pleurotus ostreatus 2191* was used in amount of 5% (a difference is slightly higher than 1%, which is unreliable). Live weight dynamics during the I test is clearly seen in Diagram №1.

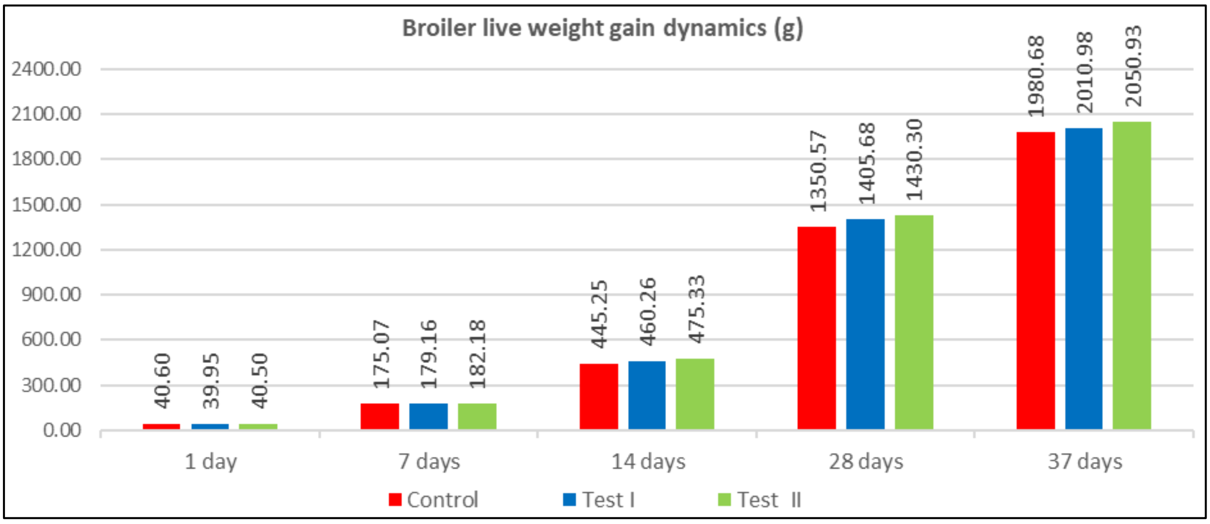

Figure 1 Production test - Diagram №1

During the second production test, as is seen from Diagram №2, five groups (one control and four test ones) have been exposed to test. Proceeding from the results obtained during the first production test, a percentage/dosage of both medicinal mushrooms *Ganoderma Lucidum 447* and *Pleurotus ostreatus 2191* in broiler's compound feed were reduced down to 2,4%. Number of broilers tested was the same for all groups, both control and test ones (100 birds in each group). Broilers of the control group, similar to a case of the first production test, in the second test period also took for first 1st-5th days the medicinal preparation / antibiotic (Enrofloxacin) along with water. As is seen from the Table, in all five groups, in the beginning of tests, a live weight of one-day broiler was nearly identical and varied between 38.84-41.00 grams, while variation coefficient was evened and varied within 2.6-3.1%.

On the 14th day of breeding a live weight of broilers of the control group comprised 444.43 grams, while that of broilers of the test groups – 470.88-478.64 grams, which is 5.9% - 7.7% higher compared to the live weight of broilers of the control group ($P \geq 0.01$), as for variation coefficient, it varied between 0.51-2.39 %.

On the 28th day of production, a live weight of broilers of the control group comprised 1295.88 grams, while in the test groups, in particular, where the medicinal mushroom *Ganoderma Lucidum* was used in amount of 2-4% (the first and second test groups), the live weight of broilers comprised 1353.13-1346.32 grams, while when using 2-4% of medicinal mushroom *Pleurotus ostreatus* (in the third and fourth test groups), broiler's live weight comprised 1356.04- 1347.42 grams, which is 50-55 grams higher that a live weight of broilers from the control group.

In the end of production, at 36-days age, the highest values of live weight have been recorded among broilers of the third test group – 1909.69 grams, which is 2.9 % higher compared to broilers of the control group ($P \geq 0.01$-0.05), while variation coefficient varied between 0.70-1.22%.

As for application of 2-4% of medicinal mushrooms *Ganoderma Lucidum* and *Pleurotus ostreatus* in broilers' compound feed, the live weight is nearly identical for all tests, and there is no relevant difference in their live weight for any rearing period. If we compare their addition to compound feed in amount of 2 and 4 percent, the live weight is almost the same in all age periods, and the difference is roughly 5-1 grams. Dynamics of live weight during the second production test for greater clarity is given in Diagram №2.

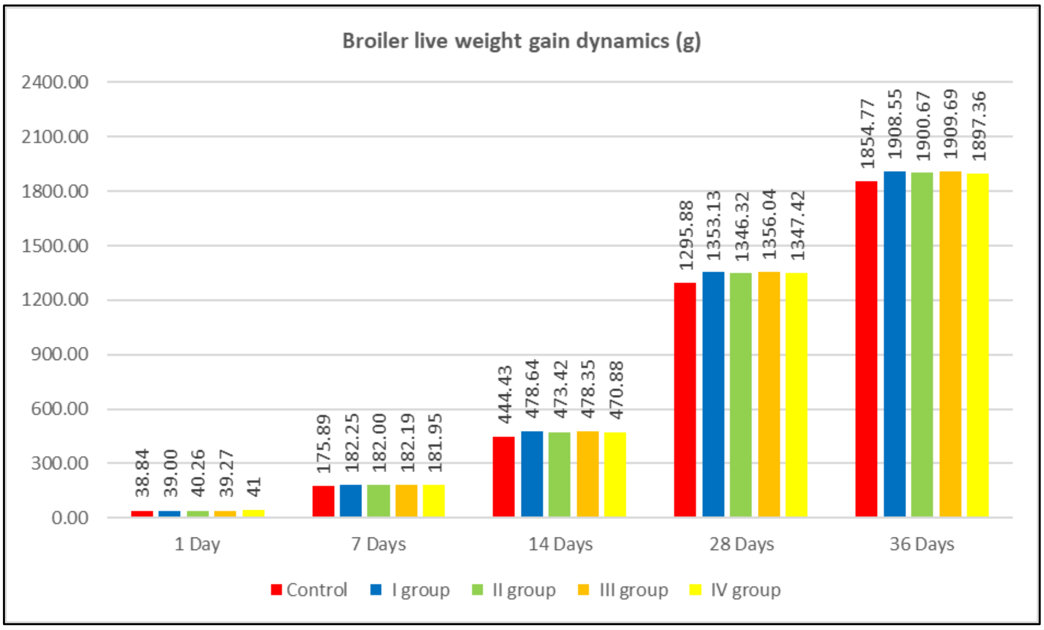

Figure 2 Production test – Diagram №2

During the third production test, three groups (one control and two test ones) were exposed to test, similar to the first and second production tests, broilers were fed by a compound feed and during the first 5 days took medicinal preparation – Enrofloxacin along with water. As for the I test group, we added to main compound feed the medicinal mushroom *G. Lucidum* 447-1% *and P. ostreatus* 2191- 1 % as a feed additive, while in case of II test group *G. Lucidum* 447-2% + *P. ostreatus* 2191- 2% were added according to experimental design.

In the beginning of a trial the live weight of one-day broiler chickens was the same in all three groups and varied within limits of 40.33-42.04 grams. The mentioned index points at the high homogeneity of one-day broiler chickens exposed to test.

On the 14th day of production, a live weight of broilers from the control group comprised 450.26 grams, while broiler weights from test groups - 507.38 - 531.94 grams, which is 12.6%-18.1% higher compared to broilers of the control group ($P \geq 0.01$-0.05), as for variation coefficient, it varied between 1.05-4.64 %.

On the 28th day of production the live weight of broilers from the control group was equal to 1362.91 grams, which is 3-5% lower, than the same parameter for broilers of the second and third groups.

At the end of production at 36-days age, the highest value of live weight was recorded among broilers of the first test group 1963.59 grams, which is 5.3 % higher compared to live weight of broilers from the control group ($P \geq 0.05$), and variation coefficient varied between 0.81-2.48 %.

Broiler’s live weight dynamics during the third production test for illustrative purposes is given in Diagram №3.

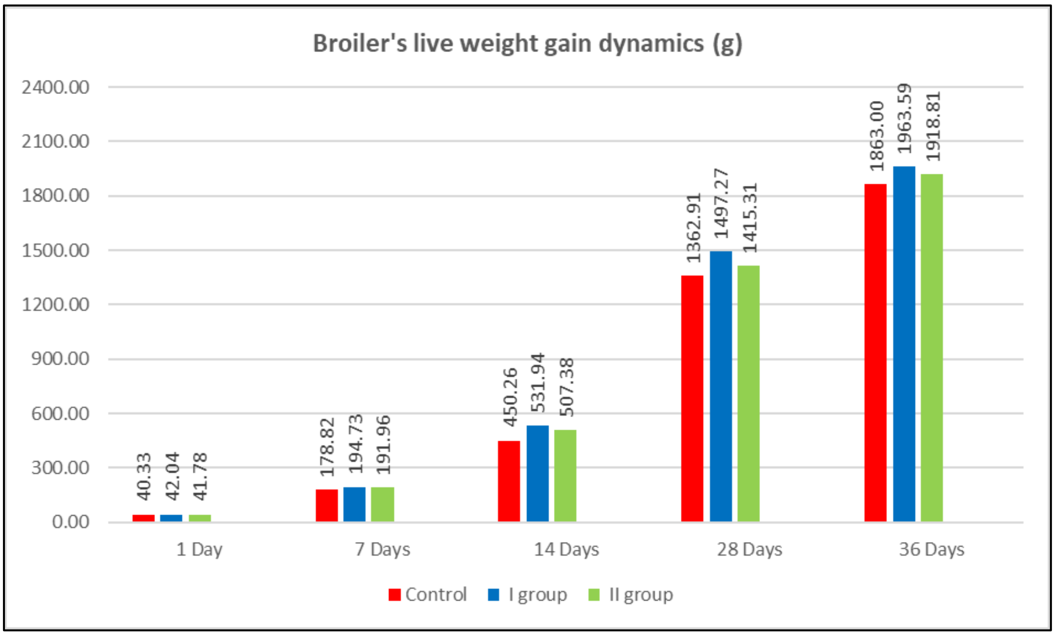

Figure 3 Production test - Diagram №3

For the fourth production test, 300 one-day chickens were selected according to principle of analogies, and they were divided by us into 3 groups (control, I test group, II test group) – 100 birds in each one.

Broilers from the control group of the fourth production test, similar to first three production tests broilers were fed by a compound feed, to which Enrofloxacin – antibiotic used by the above-mentioned poultry farm was added. As for I test group, the medicinal mushroom - *G. Lucidum* 447-1% *and P. ostreatus* 2191- 1 % + probiotic *B. subtilis* - 1% were added to the main wholesome combined feed, while in case of II test group – two percent of medicinal mushroom – 1*% G. Lucidum* 447-2% *and P. ostreatus* 2191- 2% + probiotic *B. amyloliquefaciens* - 1%.

As is seen from the first table, during the fourth production test, a live weight of broiler chicken is the same in all three groups and varies within limits of 40.61-42.16 grams. The mentioned index is a confirmation of high homogeneity of one-day broiler chicken exposed to test.

At the 14-day age, a live weight of broilers from the control group comprised 460.76 grams, while the same index of the test groups – 542.64-567.69 grams, which is 17.7%-23.2% higher compared to poultry of the control group ($P \geq 0.01$-0.05), as for variation coefficient, it varied within 0.5-6.62%.

On the 28th day of upbringing, a live weight of broilers from the control group comprised 1374.09 grams, which is 1% less, than the live weight of the second and third test groups.

At the end of breeding, at 36-day age, the highest index of live weight was recorded among broilers of the first test group - 2026.20 grams, which is 7.9% higher compared to broilers of the control group ($P \geq 0.01$), while variation coefficient varied within 0.81-2.41%.

Broiler's live weight dynamics during the fourth production test for greater clarity is given in Diagram №4.

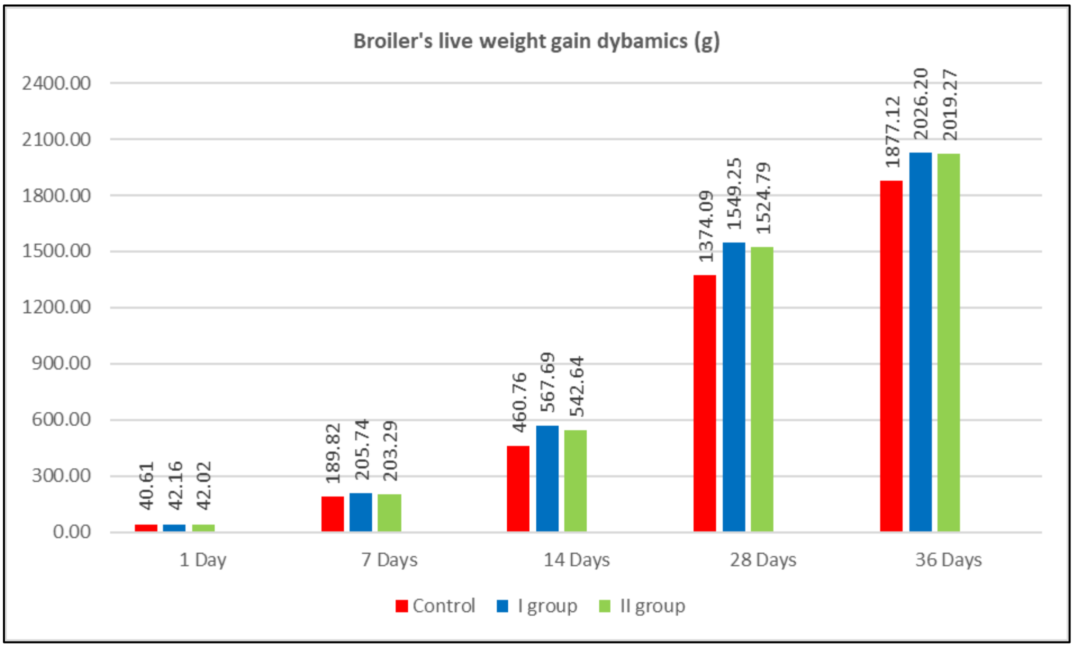

Figure 4 Production test – Diagram №4

3.2. Survival rate

Regarding broiler's survival rate, which is one of the basic parameters in terms of economic conditions of the enterprise, the following data have been registered. In the course of I production test broiler's survival rate comprised 94%, which is 1% lower index compared to data of the test group. This fact confirms reasonability of medicinal mushroom addition (in form of *G. Lucidum* 447 and *P. ostreatus* 2191) to broiler's feed ration.

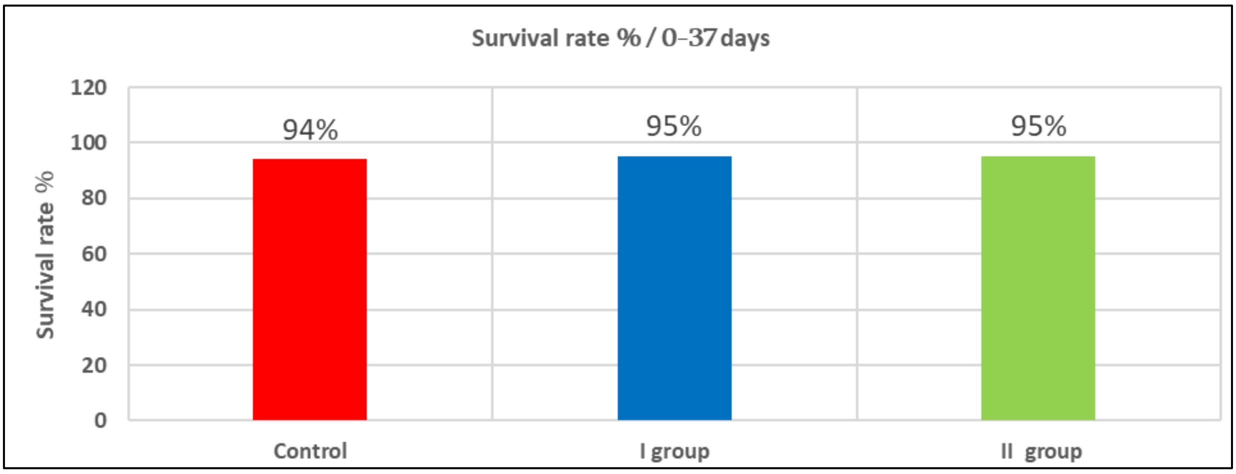

Figure 5 Production test - Diagram №12

In the course of II production test, broiler's survival rate comprised 93% for the control group, while 92-96% were registered in the test groups. Among test groups, the highest index was registered in I and III test groups – 96-96%, where 2% of medicinal mushroom *G. Lucidum* 447 and *P. ostreatus* 2191 was added as feed additive to wholesome combined feed, which is 3% higher index compared to the control group. The mentioned fact is another confirmation of reasonability of addition of medicinal mushroom *G. Lucidum* 447 and *P. ostreatus* 2191 as feed additive in such doses to broiler's feed ration.

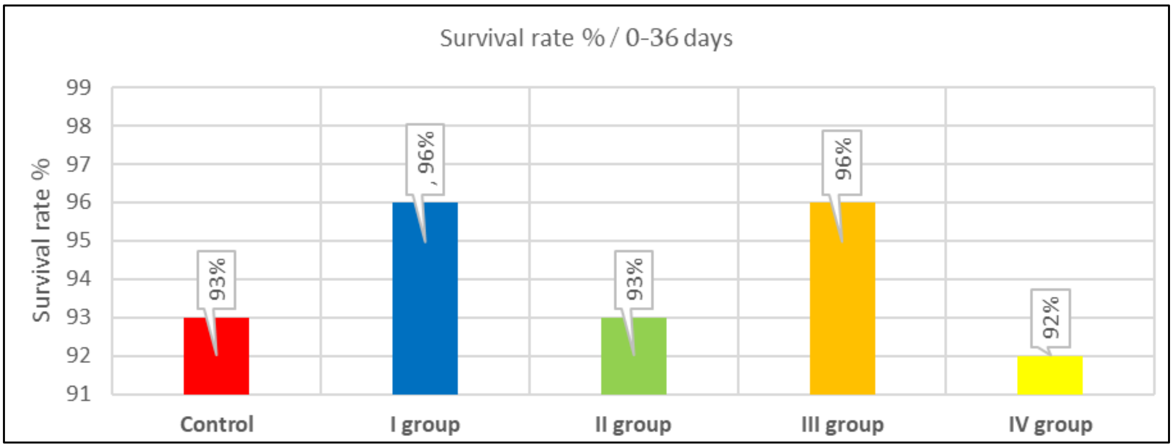

Figure 6 Production test – Diagram №13

In the course of III production test, broiler's survival rate in the control group comprised 91%, while in the test groups 93-96% was registered. Among test groups, the highest survival rate was registered in I test group – 96% (where *G. Lucidum* 447-1% + *P. ostreatus* 2191- 1 % was added as a feed additive to a compound feed), which is 5.4 % higher index compared to the control group. The mentioned fact is another evidence of reasonability of addition of such doses of medicinal mushrooms *G. Lucidum* 447-1% + *P. ostreatus* 2191- 1 % to broiler's feed ration.

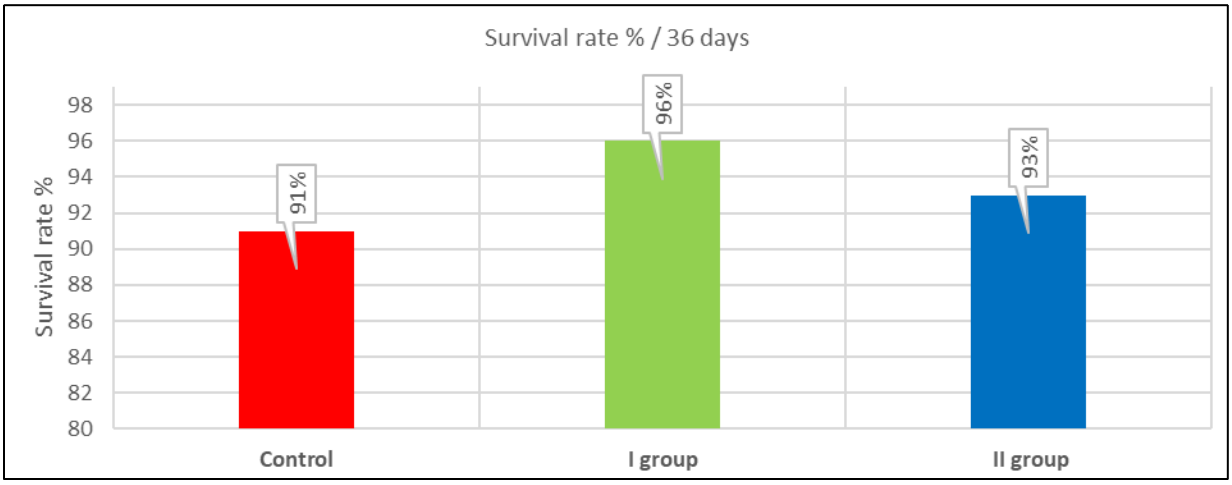

Figure 7 Production test - Diagram №14

In the course of IV production test, broiler's survival rate in the control group comprised 93%, while in both I and II test groups – 96%, which is 3.2% higher than the control group broiler's survival percentage.

Obtained result is another confirmation of advantage of joint use of the mentioned dose of medicinal mushrooms (*Ganoderma Lucidum* 447 and *Pleurotus ostreatus* 2191) and probiotic cultures (*B. subtilis* and *B. amyloliquefaciens*) as feed additive in the test group broilers' feed ration, compared to the control group broilers.

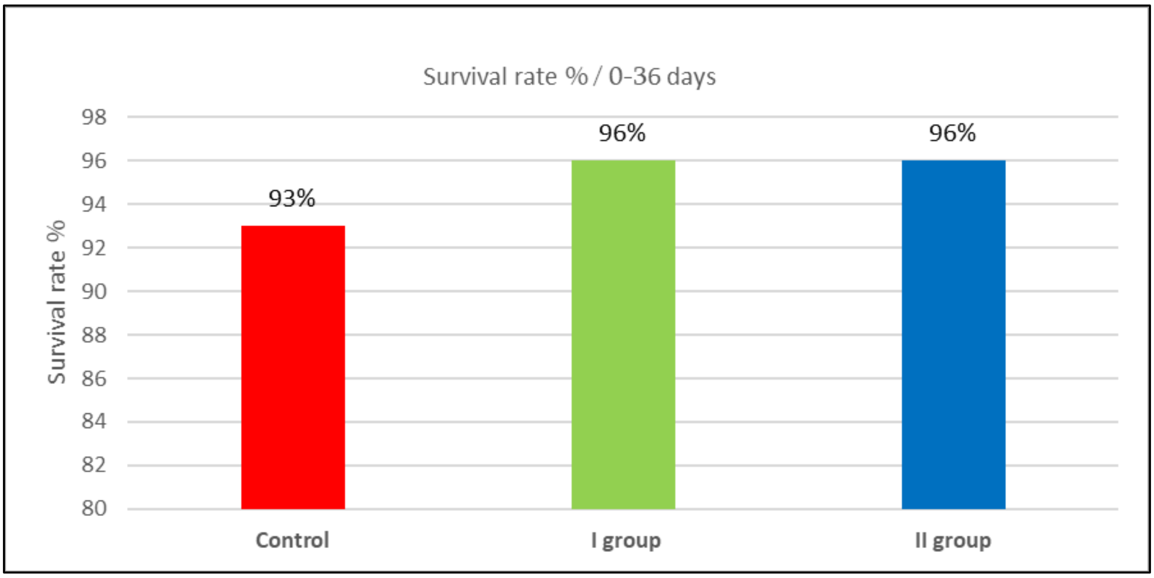

Figure 8 Production test – Diagram №15

3.3. Feed consumption

During broilers' production, one of the main parameters of broiler's productivity is feed consumption, which is calculated as a quantity of meal physically consumed both per one bird and per one kilogram of weight gain. In the course of all four tests, my means of everyday accounting of feed we have studied a quantity of meal consumed both per one bird and per one kilogram of weight gain and the results are given in Table №2.

Table 2 №2. Feed consumption during production period

Test I			
	0-37-day live weight (g)	feed consumption per one boiler (kg)	feed consumption per one kg of weight gain
Control	1980.68	3,328	1.68
I group	2010.98	3,238	1.61
II group	2050.93	3,261	1.59
Test II			
	0-36-day live weight (g)	feed consumption per one broiler (kg)	feed consumption per one kg of weight gain
Control	1854.77	3,135	1.69
I group	1908.55	3,054	1.6
II group	1900.67	3,155	1.66
III group	1909.69	3,075	1.61
IV group	1897.36	3,112	1.64
Test III			
	0-36-day live weight (g)	feed consumption per one broiler (kg)	feed consumption per one kg of weight gain
Control	1863	3.111	1.67

I group	1963.59	3.18	1.62
II group	1918.81	3.14	1.64
Test IV			
	0-36-day live weight (g)	feed consumption per one broiler (kg)	feed consumption per one kg of weight gain
Control	1877.12	3,078	1.64
I group	2026.2	3,222	1.59
II group	2019.27	3,251	1.61

In the course of I production test, feed consumption per each 1 kg of weight gain obtained was nearly the same both in the control and test groups and was recorded from 1.59 to 1.68. Though, an index of feed consumption growth in the control group was noticeable anyway. One can see the data in Diagram №16.

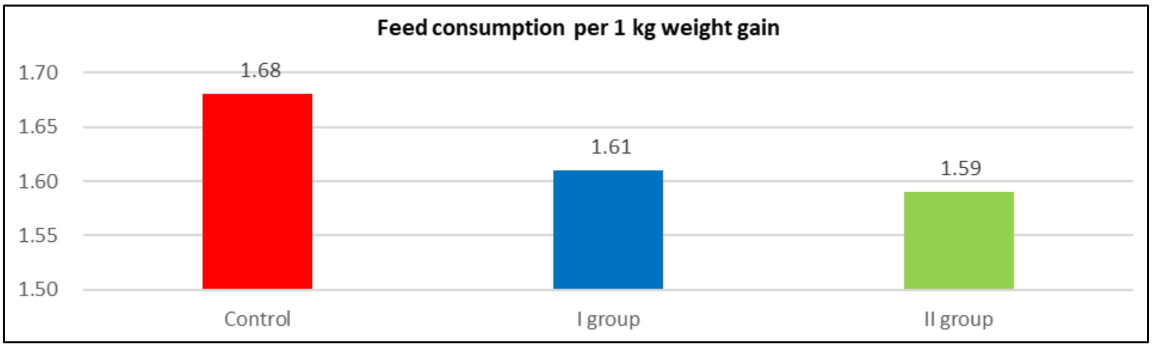

Figure 9 Production test - Diagram №16

During the II production test, nearly identical results have been obtained per each 1 kg weight gain obtained both for the control and test groups – from 1.60 to 1.69. Though, similar to the I production test, the index of feed consumption growth has been observed when growing broilers of the control group in regard to the same index of the test group broilers. Data may be seen in Diagram №17.

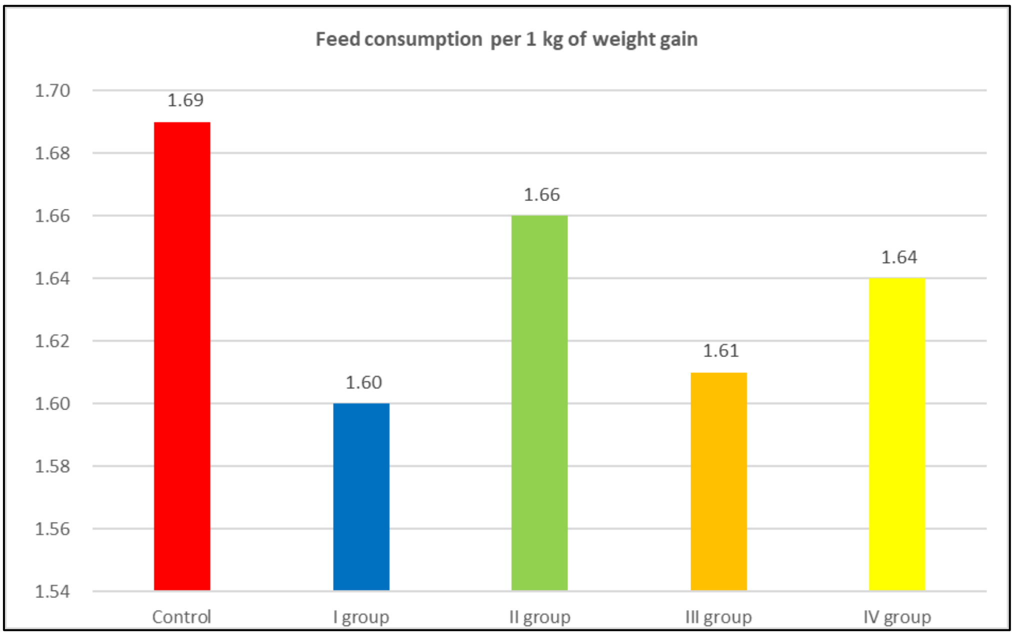

Figure 10 Production test - Diagram №17

In the course of III production test, almost the same results have been registered both for the control and test groups – from 1.62 to 1.67 per each 1 kg of weight gain. Though, similar to the II production test, the index feed consumption growth has been observed when breeding broilers from the control group in regard to the same index of broilers from the test group. Data may be seen in Diagram №18.

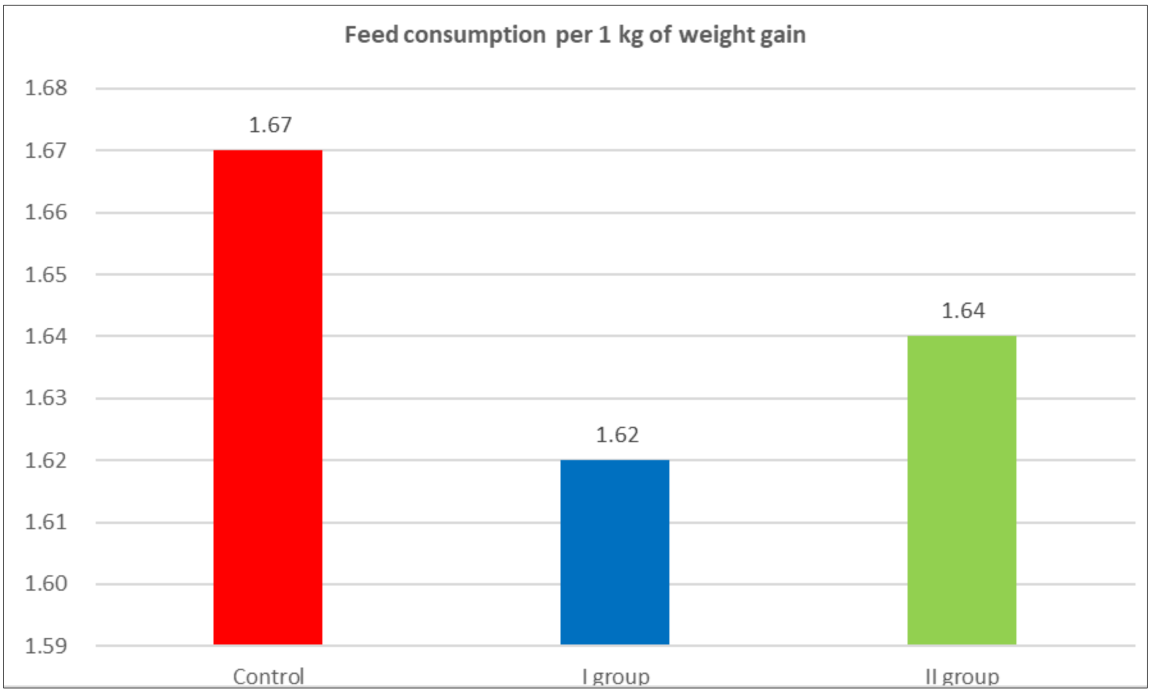

Figure 11 Production test - Diagram №18

During the IV production test, nearly identical results, from 1.59 to 1.64, have been obtained for each 1 kg of weight gain both for the control and test groups. Though, similar to the I production test, the index feed consumption growth has been observed when breeding broilers from the control group in regard to the same index of the test group broilers. Data may be seen in Diagram №19.

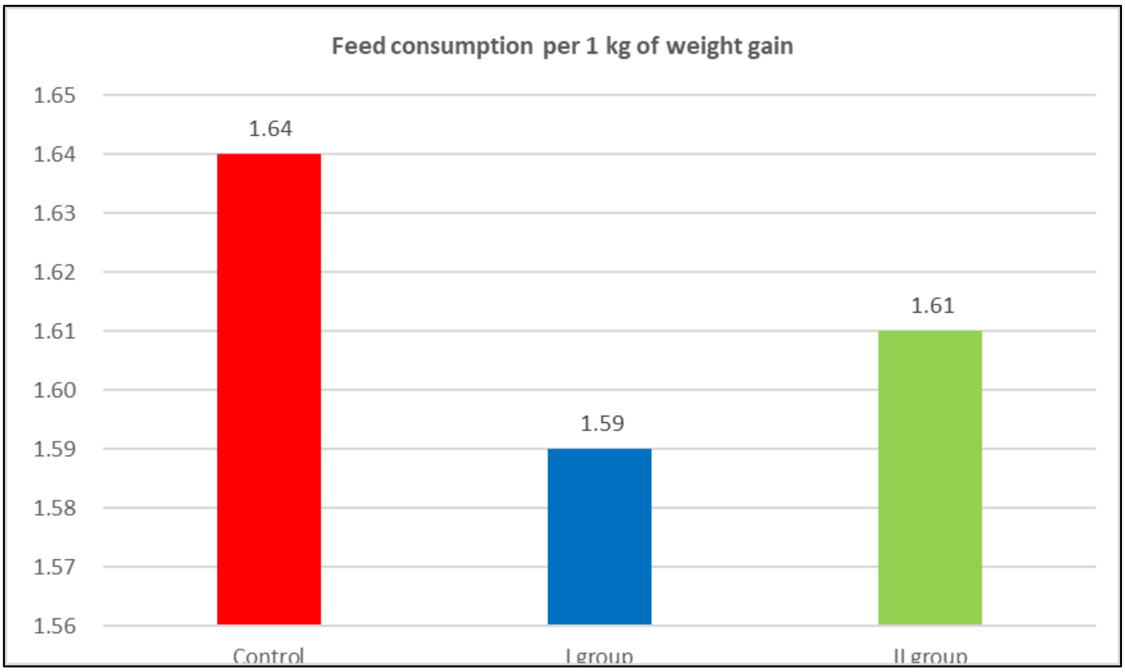

Figure 12 Production test - Diagram №19

3.4. Productivity index

Broiler meat production under factory conditions is assessed in terms of productivity index and points at the level of efficiency of broiler meat production. This index actually combines four main parameters (live weight, survival rate, feed conversion and slaughter age). In the course of tests, we have assessed the final results for one or another group in terms of this index, which are given in Diagram №20.

During the I production test, a productivity index is nearly identical to each other among broilers of the test groups and comprises 321-331, though this index of both test groups at least by 21 units and maximum by 31 units exceeds that of broilers from the control group.

Productivity indicators for poultry (broilers) have been calculated according to European index formula:

$$\text{European index} = \frac{live\ weight * survival\ rate\ (\%)}{\text{feed conversion} * slaughter\ age\ (\text{days})} * 100$$

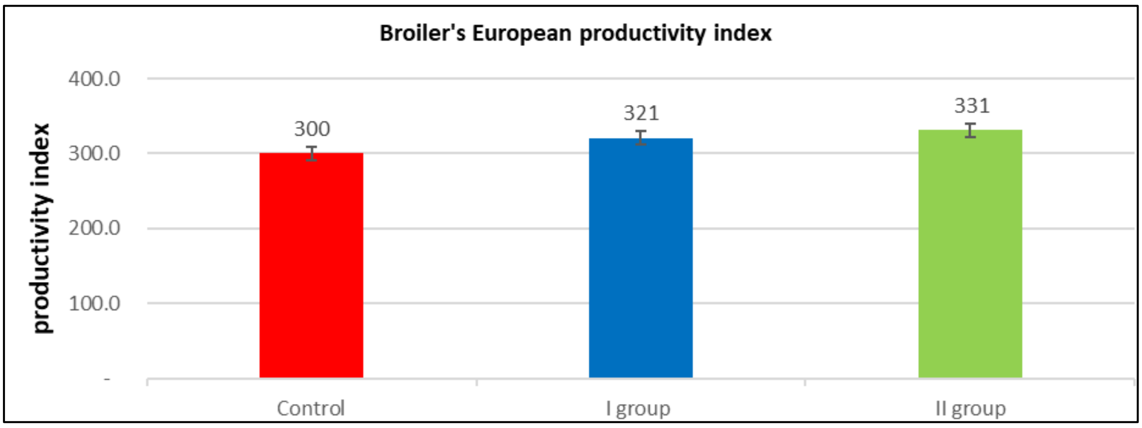

Figure 13 Production test - Diagram №20

In the course of II production test, a productivity index is nearly identical to each other among broilers of all test groups and comprises 296-318, though this index of both test groups minimum by 13 units and maximum by 35 units exceeds the figures of broilers from the control group.

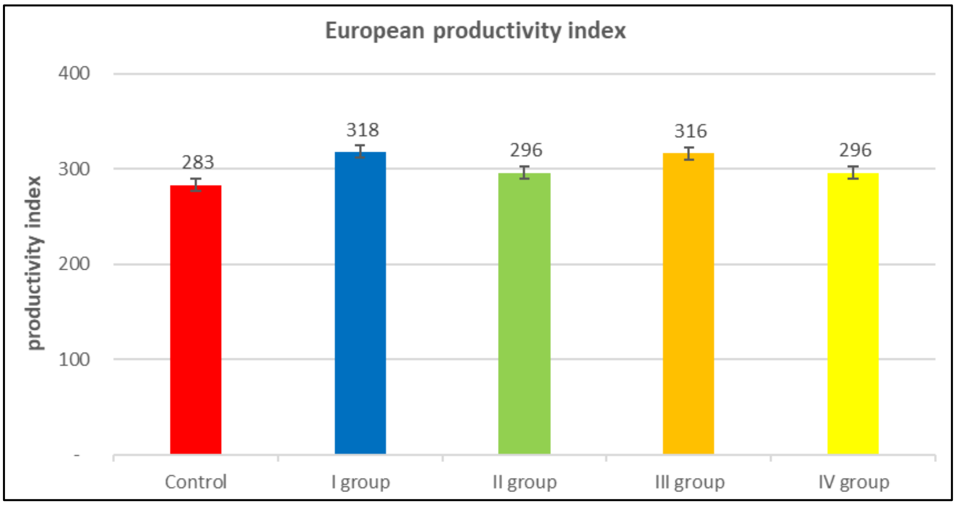

Figure 14 Production test - Diagram №21

During the III production test, a productivity index is nearly identical to each other among broilers of all test groups and comprises 302-323, and data of the test groups are different compared to the control group and are higher by 20-41 units. See Diagram №22.

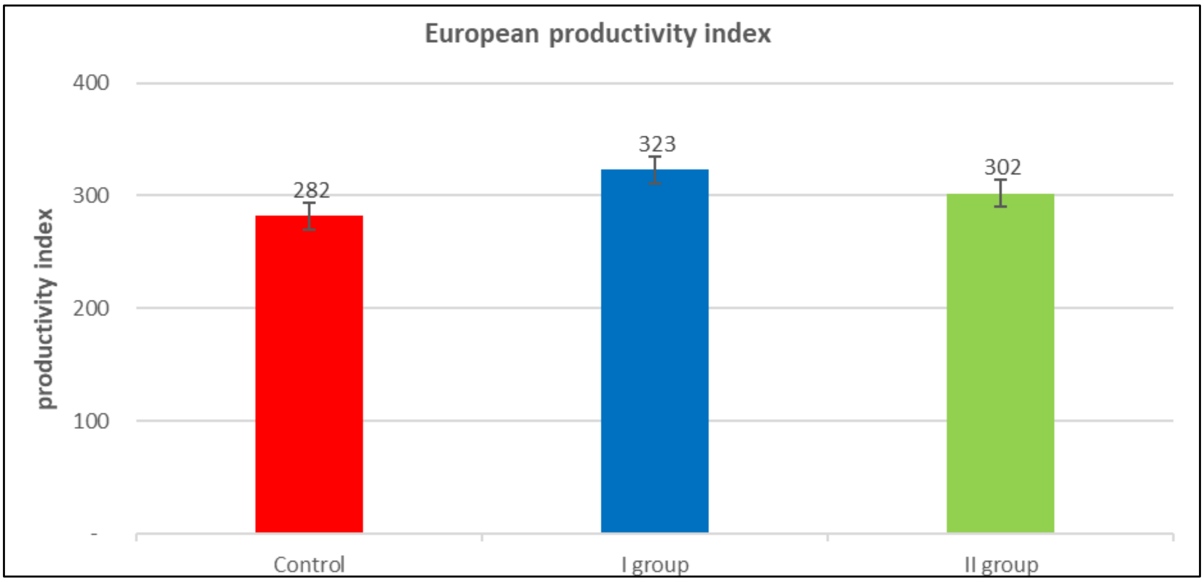

Figure 15 Production test - Diagram №22

The productivity index of broilers of the test groups during the IV production test varies between 334-339, while among broilers of the control group this parameter is equal to 295. Data obtained for the test groups are higher by 39-44 units compared to the control group. See Diagram №23.

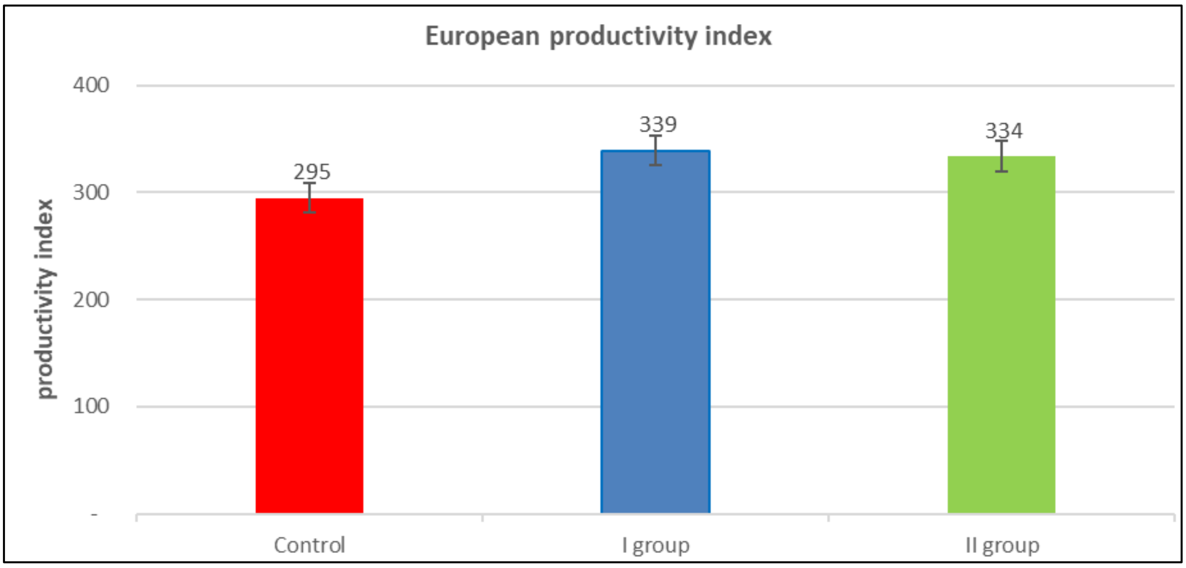

Figure 16 Production test - Diagram №23

4. Conclusion

The carried-out tests have illustrated that an application of medicinal mushrooms and probiotics in broilers' compound feed ration provides positive results. Live weight gain, feed conversion and survival rate indices have been improved in the test groups, which is in compliance with the trends recorded in the international studies. As a result, the mentioned feed additives may be considered as a safe, economically feasible and effective alternative to antibiotics, which make substantial contribution to poultry productivity growth and food safety provision in the local poultry farming.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed. The authors declare that they have no conflict of interest to disclose.

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