Application of a Probiotic Based on *Bacillus Subtilis* for Broilers

Mamatova Zamira Baratovna¹; Nasimov Shuhrat Naimovich^{1*}; Yuldasheva Madina Kaxramonovna²; Safarov Xurshid Abdurajabovich³

¹Candidate of Veterinary Sciences, Department of Diseases of Fowl, Fish, Bees and Fur Animals, Samarkand Institute of Veterinary Medicine, Samarkand, 140103, Uzbekistan; ²Department of Veterinary Surgery and Obstetrics, Samarkand Institute of Veterinary Medicine, Samarkand, 140103, Uzbekistan; ³Ph.D. candidate, Research Institute of Veterinary Medicine, Tayloq district, Samarkand region, 141500, Uzbekistan

*Correspondence: Nasimov Shuhrat Naimovich, Candidate of Veterinary Sciences, Department of Diseases of Fowl, Fish, Bees and Fur Animals, Samarkand Institute of Veterinary Medicine (SamIVM), Samarkand, 140103, Uzbekistan. E-mail: <u>nasimovshuxrat@gmail.com</u> An abbreviated running headline: Appl. prob. based B.sub. broilers

Abstract

The article was aimed at the effectiveness of using the local probiotic to improve the productivity of broiler chickens. Testing the effect of the drug was carried out on day-old broiler chickens of the cross Ross-308. 0,5 and 1,5 million of probiotic microorganisms were given with drinking water during the observation period in the first experiment, 100 and 200 million microbic bodies – in the second. The following indicators were taken into account: the safety of the livestock, the live weight of the chickens. According to the results of the experiments, the positive effect of the probiotic (profit 121%) on the productivity of broiler chickens in relation to the chickens of the control group was determined. A priority method of supplying probiotic was established, as well as a possible daily dose of probiotic for broiler chickens (200 million microbial bodies). The safety of the livestock in the groups that received antibiotics and probiotics was 102%, in relation to the control group (1 dead). The probiotic "PP-1" prepared by us is effective for increasing the productivity of broiler chickens; it is safe for health and can be used instead of antibiotics in poultry for the prevention of infections;

Key words: chickens, probiotics, livestock, Uzbekistan, Bacillus, water

Introduction

Poultry farming is an important and fast-growing branch of agriculture of Uzbekistan. The task of increasing the profitability of poultry farming dictates the needs to obtain high-quality and environmentally friendly products. However, at present, in the conditions of developing poultry farms, there is a complex of adverse factors that threaten the health of poultry and cause stress on it:

1. These are, first of all, infections, inadequate feeding, toxic and microbial contamination of feed, unfavorable conditions of keeping, leading at first to an imbalance of the intestinal microflora, which reduces the body's resistance, and subsequently leads to the death of poultry.

2. Constant therapeutic and prophylactic measures - antibiotic therapy, vaccination, which have not only a positive (therapeutic), but also a negative effect in the form of selection and circulation of pathogenic and opportunistic microorganisms, which have become resistant because of

many years of use of antibiotics in farms, which, in turn, cause a wide spread of diseases that become stationary in farms and cause the death of poultry.

According to the theory of academician A. Ugolev, digestion is a two-phase process: chemical, carried out by a number of enzymes and acids; and microbiological – food, after entering the body, starting from the oral cavity and further in the stomach and intestines, is exposed to various microorganisms, which are representatives of the normal microflora of the body that helps break down nutrients from complex biological ones to easily digestible and absorbed. The microbiological process is normally 40% of the total digestion.

The intensification of poultry farming determines the poultry in a closed, limited space, especially with cage keeping, which leads to a lack of contact with natural donors of beneficial microorganisms (soil, insects, plants). Monotonous feeding aimed at obtaining maximum production, a lack of essential proteins, vitamins, minerals in the diet of poultry leads to the colonization of the chickens' intestines with pathogenic microorganisms due to the normal microflora of the gastrointestinal tract. As a result, the microbiological balance is disturbed, leading to changes in digestion and absorption, the imbalance is aggravated, leading to a number of gastrointestinal diseases, nonspecific resistance decreases, the susceptibility to various infectious diseases increases, at best, the mass of internal organs and muscles decreases, which prevents normal growth and development, and subsequently, productivity.

Currently, in world practice, to stimulate the growth and prevent gastrointestinal diseases, probiotics and prebiotics are used, which contribute to the colonization of the intestines with their own bifidoflora, suppressing pathogenic bacteria, enhancing the absorption of nutrients, activating the body's defenses (Donnik and Lebedeva, 2011; Khaksefidi and Ghoorchi, 2006).

Probiotics, being cultures of microbes that are symbiotic to the normal microflora of the gastrointestinal tract, suppress the vital activity of pathogenic and opportunistic intestinal bacteria, increase the body's resistance, improve the absorption of nutrients in feed, and activate metabolic processes. Probiotics create a positive balance of the microflora of the digestive tract by populating it with competitive strains of probiont bacteria that exercise nonspecific control over the number of opportunistic microflorae by displacing it from the intestinal microbiocenosis (Denisov, 2009; Donnik and Lebedeva, 2011; Fox, 1988).

Probiotics are used to stimulate nonspecific immunity, prevent and treat mixed gastrointestinal infections, digestive disorders with alimentary etiology - dysbacteriosis resulting from a sharp change in the composition of the ration, violation of feeding and maintenance regimes, technological stress and other reasons; changes in the microflora of the digestive tract after treatment with antibiotics and antibacterial agents; replacing antibiotics in compound feed for young animals; improving digestion processes; increasing the efficiency of feed use in animal productivity (Egorov and Kuzmenko, 2014; Kornienko and Ulitko, 2014; Barrow, 1992; Khajali, 2008).

It has been established that the use of probiotics can have an anti-infectious, immunomodulatory effect on the body, increase barrier functions that protect from the action of the environment, prevent the penetration of pathogenic microflora into the body, stimulate motility and intestinal secretion (Danilevskaya, 2006; Kaloev and Ibragimov, 2017; Karsyukov, 2004; Fuller, 1989; Tohtiyev, 2009; Shirshikov et al., 2007; Tarakanov, 2000).

However, at present, a situation has developed in Uzbekistan that is favorable for the import of probiotic drugs. The growth in imports of veterinary probiotics entails additional expenses for poultry farmers, including not only the cost of obtaining drugs, but also customs and transportation costs.

1. Taking into account the multifaceted positive effect of probiotics on the body of fowls and the lack of local drugs on the Uzbek market, we set the goal of our research to test the effectiveness of the probiotic prepared by us to increase the safety and productivity of broiler chickens.

The research tasks were:

1. Preparation of a laboratory sample of probiotic for testing on broiler chickens;

2. Test of the probiotic prepared by us to increase the safety of livestock and productivity of broiler chickens;

3. Determination of the method of giving and the dose of the probiotic prepared by us for broiler chickens.

Materials and methods

Live microbes of probiotic *Bacillus subtilis* from samples of meadow forbs obtained by us at the Department of Diseases of Fowl, Fish, Bees and Fur Animals of the Samarkand Institute of Veterinary Medicine were taxonomically identified by microscopic, microbiological and biological methods in the Bacteriological Department of the State Center for Food Safety and Diagnosis of Animal Diseases of Samarkand Region. The number of microbial bodies in 1 ml of probiotic was determined using an optical turbidity standard and in our further studies we used doses of 0.5 and 1.5 million, 100 and 200 million microbial bodies. Experiments to determine the effectiveness of the use of the probiotic, which we named "PP-1", were carried out in the SamIVM poultry house.

The test of the effect of the probiotic in the first experiment was carried out on selected dayold broilers of the cross Ross-308, which were divided into 3 groups - one control and two experimental, 10 heads each. The chickens of the control and experimental groups were given the same conditions of keeping in accordance with zoo hygienic requirements, feeding was carried out with pelleted feed in the first 10 days - "Start", the next 10 days - "Growth" and until slaughter -"Finish".

In the first experiment, 0.5 million microbial bodies of the probiotic were given with a pipette every day during the entire period of growing to the chickens of the first experimental group, to the second experimental group - 1.5 million microbial bodies.

In the second experiment, 100 and 200 million microbial bodies of the probiotic were given with drinking water during the first 10 days.

During the experiment next indicators were considered: safety of livestock, live weight of chickens (every 10 days) during 35 days of observation. Results of the first experiment presented in Table 1.

Results

From the data shown in Table 1, it can be seen that the probiotic drug "PP-1" prepared by us gives 100% safety of the livestock of chickens and has a positive effect on the increase in live weight of broiler chickens (2309 g per head in the group that received 1.5 million microbial bodies per day, which is 104% in comparison to the control group of chickens that had an increase in live weight 2220 g per head for 35 days of observation). The first experimental group of chickens receiving 0.5

million microbial bodies of the probiotic per day showed low performance in relation to the chickens of the control group.

Average daily gain in the control group is 70,1+1,5, in the first experimental group - 67,8+0,95 (96 % in relation to control group), in the second experimental group - 73,13+1,20, which is 104%.

The data of the economic efficiency of the experiment is presented in Table 2.

At the time of the experiment 1 USD = 9539 UZS (as February, 11, 2020, Central Bank of Uzbekistan). The economic efficiency of the probiotic prepared by us in view of very small doses of probiotic and severe stress for chickens, which had to be picked up and watered every day, the probiotic is not very well traced on the example of data from the first experimental group of chickens. In chickens that received a large dose of the probiotic (the second experimental group) at a feed and probiotic cost of 14.01 thousand UZS, an increase of 23.09 kg was observed (at a cost of 1 kg of chicken, 14000 UZS), while in the control group, the cost of feed and probiotic was amounted to 13.2 thousand UZS (difference -0.81 thousand UZS) with an increase of 21.05 kg (the difference was 23.09-21.05 = 2.04 kg). Thus, having spent an additional 0.81 thousand UZS, we received 2.04x14000 = 28.56 thousand UZS of profit in the group, 2.8 thousand UZS for 1 head respectively, the profit for 1 spent UZS in relation to the control group was 108%.

The results of the effect of the probiotic in doses of 100 and 200 million microbial bodies on the safety and growth of chickens (groups 3 and 4) in the second experiment were also studied in 1 to 35 days old chickens of the cross Ross-308. The chickens of the second experimental group were given enrofloxacin, which is used in poultry farms for the prevention of infectious diseases; the chickens of the control and experimental groups were not given any preventive measures. Each group had 20 chickens. The probiotic was given with water for 10 days. The experimental results are presented in Table 3.

According to the results of the second experiment, the goals of which were to determine the method of giving and the dose of the probiotic to chickens, as well as the possibility of replacing antibiotics with probiotics, there are a positive effect of the probiotic (121% profit) on the productivity of broiler chickens in relation to chickens of the control group (100%) and chickens receiving antibiotics in a prophylactic dose (100%).

In the second experiment, the priority of feeding the probiotic drug with drinking water was established (during the day, probiotic microorganisms do not die and remain effective, the chickens are not exposed to excessive stress, the possibility of chicken contamination upon contact with the staff is excluded), as well as a possible daily dose of probiotic for broiler chickens (200 million microbial bodies).

The safety of the livestock in the groups that received antibiotics and probiotics was 102% in relation to the chickens in the control group (1 dead chicken).

	Indicators	1-Control group	2-Experimental group	3-Experimental group
a t o	5 days old	115	116	115
Live eight,	10 days old,	323	329	333
L wei	% to control group	100%	101%	103%

Table I. Results of the effect of the probiotic "PP-1" on the safety and growth of chickens

International Journal of Future Generation Communication and Networking Vol. 14, No. 1, (2021), pp. 4962 – 4969

20 days old,	852	980	975
% to control group	100%	115%	114%
35 days old,	2220	2150	2309
% to control group	100%	96%	104%
Average daily gain, % to	70,1+1,5	67,8+0,95	73,13+1,20
control group	100%	96%	104%
Safety of livestock,	10	10	10
%	100%	100%	100%

Table II. Economic evaluation of the results of the experiment

Indicator	1-Control	2 Experimental group	3-Experimental
mulcator	group	2-Experimental group	group
Feed, kg per head	3,3	3,3	3,3
Total feed and probiotic	13200	14000	14010
cost, UZS	100%	106%	106%
Live weight gained, g	2150	2220	2309
	100%	103%	107%
Group live weight	30,1	31,08	32,349
gained, kg	100%	103%	107%
Costs per 1 kg of gained	6139	6306	6067
live weight, UZS	100%	105%	111%
Profit per head	16900	17080	18339
	100%	101%	108%
Efficiency per 1 UZS	2,28	2,22	2,3
spent	100%	98%	100%

Table III. Results of the effect of the probiotic "PP-1" on the safety and growth of chickens

	Indicator	1- Control group	2-Experimental group	3- Experimental group	4-Experimental group
	7 days old	150	145	154	155
50	14 days old,	450	442	455	453
weight,	% to control group	100%	98%	101%	100%
Live	21 days old,	867	870	895	910
Ľ	% to control group	100%	100%	103%	104%

28 days old,	1400	1500	1500	1550
% to control group	100%	107%	107%	110%
35 days old,	1950	2075	2180	2230
% to control group	100%	106%	111%	114%
Average daily gain,	64,28	68,92	72,35	74,10
% to control group	100%	107%	113%	115%
Safety of livestock,	39	39	40	40
% to control group	100%	100%	102%	102%

	1-	2-	3-	4-
Indicator	Experimental	Experimental	Experimental	Experimental
	group	group	group	group
Feed, kg per head	3,3	3,3	3,3	3,3
Total cost of feed and	13200	14050	14100	14110
probiotic, UZS				
Live weight gained, g	1950	2075	2180	2230
	100%	106%	111%	114%
Group live weight	27,3	29,05	30,52	31,22
gained, kg	100%	106%	111%	114%
Costs per 1 kg of gained	6769	6771	6467	6327
live weight, UZS	100%	100%	96%	93%
Profit per head, UZS	14100	15000	16420	17110
	100%	106%	116%	121%
Efficiency per 1 UZS	2,06	2,06	2,16	2,21

Discussion

The positive effect of probiotics in poultry and the lack of local drugs in the market of Uzbekistan were the main reasons of this research.

As results, we managed to create a probiotic based on *B. subtilis* from herbs from the mountain and foothill areas of the Republic.

Nowadays, probiotics are widely used in poultry industry as biologic supplements in nutrition in order to boost the role of normal intestinal bacterial biocenosis and to reduce the negative impact of antibiotics on microecological system of organism. As body muscle increases rapidly and disproportionate mass decrease of internal organs occurs, which leads to weakening the general resistance of broilers, good conditions appear for opportunistic microflora. In our studies, a positive effect (profit 121%) of the probiotic prepared by us was determined on the productivity of broiler chickens in relation to chickens of the control group, which correlated with the results of a number of researchers (Fisenko, GV et al, 2015).

The use of antibiotics in poultry has a number of features, in which the main one is the impossibility of individual use. At the same time, not one bird receives the antibiotic, but all the livestock. The consequence of the use of antibiotics leads to an increase in resistance to them in people who consume poultry meat. Our research of the effectiveness of our drug against pathogenic and opportunistic microflora instead of antibiotics represented that it is relevant and the probiotic can enhance the productivity up to 121%.

At the same time, the priority was set for drinking the probiotic drug with water.

As a result of repeated studies to determine the dose of the probiotic preparation, a possible daily dose for broiler chickens was established with the amount of 200 million microbial bodies. But it still requires additional research.

The results of studying the safety of the livestock in the groups of chickens that received antibiotics and probiotics demonstrate positive indicators in relation to the control group, which indicates the possibility of replacing feed antibiotics with probiotics. In this regard, the probiotic "PP-1" can be used in poultry farms of various scales.

We believe that a detailed study of the form and timing of use, the dose of this drug will be the final stage of our work.

Acknowledgement

Authors wish to thank administration of Samarkand Institute of Veterinary Medicine for providing space for research. The authors received no financial support for the research, authorship or publication of this article.

Conflict of interests

All named authors do not have conflict of interest, financial or otherwise.

References:

1. **Barrow, PA** (1992) Probities for chickens. Probities: The Scientific Basis (Ed. Fuller R.). Chapman and Hall, London, UK. PP: 225-227.

2. Collins, MD and Gibson, GR (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am. J. Clin. Nutr., №5. PP: 1052-1057.

3. **Danilevskaya, NV** (2006) Features of probiotic drugs and the possibility of their use for animals in zoos. Feeding of the Wild Animals: Inter-department scientific and methodical articles. Moscow zoo. Moscow, Russian Federation. PP: 23-29

4. **Denisov, GV** (2009) The use of probiotics in industrial poultry. Veterinariya, №4. PP: 15-17.

5. Donnik, IM and Lebedeva, IA (2011) The condition of the stomach and intestines of broiler chickens when using the probiotic Monosporin. Veterinariya Kubani, Krasnodar, Russian Federation. N_{23} . PP: 15-16.

6. **Drozdova, LI** (2012) The influence of probiotics on the environmental aspects of the quality of poultry meat products. Himiya i zhizn – XXI vek, №1. PP: 151-158.

7. **Egorkina, NA, Loboda, II, Kovalev, VV and Korolkova, SV** (2016) The choice of probiotic and the method of studying the effectiveness of its use during stress in carp in aquariums. Ecology and bioresources, Materials of the 5th International conference of young scientists of Aquaculture Center Network in Central and Eastern Europe, 46. PP: 156-166.

8. **Egorov, BV and Kuzmenko, YY** (2014) Probiotics in poultry feeding. Hranenie i pererabotka zerna. Odessa, Ukraine. PP: 39-41.

9. Feoktistova, NB, Mardanova, AM, Xadiyeva, GF and Sharipova, MR (2017) Probiotics Based on Bacteria from the Genus Bacillus in Poultry Breeding. Uchenye zapiski kazanskogo universiteta. Seriya estestvennye nauki (Proceedings of Kazan University. Natural Sciences Series), Vol. 159, No. 1. PP: 85–107.

10. Fisenko, GV, Koschaeva, OV, Lysenko, YA (2015) Probiotics in compound feed for laying hens and broiler chickens. Molodoy ucheniy, №8(88). PP: 404-407.

11. Fox, SM (1988) Probiotics: Intestinal inoculants for production animals. Veter. Med. (Edwwardsville), № 8. PP: 806-810, 824-830.

12. **Fuller, R** (1989) Probiotics in man and animals. Journal of Applied Bacteriology, №6. PP: 365-378.

13. **Ivanova, AB and Kuznetsova, AB** (2009) The effectiveness of probiotics based on *Bacillus subtilis, Bacillus licheniformis* when growing chickens of the cross Hisex-Brown. Bulletin of Novosibirsk State Agrarian University, №11. PP: 19-24.

14. **Kaloev, BS and Ibragimov, MO** (2017) The change of biochemical counts of broilers blood when using enzyme drugs in their rations. Inter-nauka: Scientific J., N_{2} 2(6). Moscow, Russian Federation. Pub. "Internauka". PP: 52-53.

15. **Karsyukov, DV** (2004) Pharmaco-toxicological properties and application of Vitaton. Synopsis of thesis. Krasnodar, Russian Federation. PP: 28.

16. **Khajali, F, Karimi, S and Qujeq, D** (2008) Probiotics in drinking water alleviate stress of induced molting in feed-deprived laying hens Asian-Aust. J. Anim. Sci. Vol. 21, No. 8. PP: 1196 – 1200.

17. **Khaksefidi, A and Ghoorchi, T** (2006) Effect of Probiotic on Performance and Immunocompetence in Broiler Chicks. The Journal of Poultry Science, №8. PP: 296-300.

18. Kokov, T and Temrokov, A (2006) Bentonites in poultry ration. Ptitsevodstvo, №12. PP: 19-20.

19. **Kornienko, AV and Ulitko, VE** (2014) Enzyme-probiotic drug Bacell in the diets of sows as a factor in increasing the level of realization of their bioresource potential. Zootehniya, №10. PP: 8-9.

20. **Pishmantseva, N, Kovehova, N et al.** (2011) Probiotics increase poultry profitability. Ptitsevodstvo, № 2. PP: 36–37.

21. Shirshikov, NV, Redikultsev, YV, Muzafarov, EN and Gavrilov, AB (2016) Cultivation of Bacillus sublitis in the structure of a non-waste technology of probiotic veterinary drug. Izvestiya Tulskogo Gosudarstvennogo Universiteta. Estestvennie nauki, 1. PP: 99-108.

22. **Tarakanov, BM** (2000) The mechanisms of action of probiotics on the microflora of the digestive tract and the body of animals. Veterinariya, N_{2} 1. PP: 47 – 54.

23. Tohtiyev, AG (2009) Application of probiotics in poultry. Ptitsevodstvo, №12. PP: 25.