Investigation of the effectiveness of antimicrobial treatment of poultry products by electrophysical methods

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Abstract. Improved methods of processing food and agricultural products make it possible to achieve an optimal effect at an acceptable level of its quality. In radiobiology, Methods of synergistic effect of two factors are widespread, in particular, the combination of treatment with ionizing radiation (IR), UV and plasma. The use of these methods in the production cycle can significantly improve processing efficiency. In the present work were treated combined feeds of ‘PK-5’ and ‘PK-6’ in order to investigate the synergistic effect of IR treatment and high pressure gas discharge plasma (HP GDP) radiation. As the source of IR was used the nanosecond electron beam (NEB) of the accelerator URT-1 (1 Mev). To create the HP GDP was used a high-voltage nanosecond generator GVI-150 (150 kV). The obtained results indicate the presence of a synergistic effect of the combined effects of plasma radiation and NEB.

1. Introduction
Of particular interest in the modern food and agricultural industry are new methods of processing products to increase shelf life and the level of biological safety. Treatment by ionizing radiation (IR) [1], ultraviolet light (UV) [2] and plasma [3] is becoming more and more widespread. These electrophysical methods allow processing of products at a temperature close to room temperature. Radiation treatment reduces microbiological contamination of products, however, due to the presence of a dose load, the nutritional value of the products decreases. At the same time, UV treatment is ineffective and requires long exposure. In radiobiology are widespread methods of synergistic effect of two factors, in particular, the combination of IR, UV and plasma treatment.

2. Materials and methods
The source of IR was the nanosecond electron beam (NEB) of the accelerator URT-1 [4] (1 Mev, 100 ns, 50 Hz). To measure the absorbed dose (AD) were used plastic dosimeters of CO AD (E) -1/10 and CO AD (F) R-5/50. High-voltage nanosecond generator GVI-150 [4] (150 kV, 100 ns, 300 Hz) was used to create high-pressure nanosecond gas discharge plasma (HP NGDP). To vary the dose of plasma radiation was changed the duration of exposure of samples under radiation.

Feed for broilers was processed: ‘PK-5 Start’ and ‘PK-6 Finish’ (GOST 18221-2018 Complete feed for agricultural poultry. General technical specification). Complete feed ‘PK-5 Start’ is used for
chickens from the first day to 4 weeks of life, the feed is in the form of grits. Complete feed ‘PK-6’ is intended for broiler chickens aged 29 days and older, the design is granules with a diameter of 4-4.2 mm. The feed includes forage wheat, barley without shell, forage corn, corn gluten, soybean extraction cake, sunflower extraction cake, fish flour, meat-and-bone flour, soybean oil, amino acid additives. The manufacturer's recommended shelf life for feed is 3 months. Samples of feeds for the studies were taken as follows: samples of ‘PK-6’ were taken from a batch that only came to the enterprise (2 weeks from the date of production); ‘PK-5’ samples were taken from the batch during the third month of storage (3 weeks prior to the expiration of the manufacturer's recommended shelf life). Feed samples were taken from a storage bunker at a poultry plant, then stored for 14 days in sterile laboratory regulated conditions (temperature 20°C, humidity 50%). Then, the feed samples were divided into experimental and control batches, the experimental ones were treated by NEBs with AD 5, 10 and 15 kGy. After NEB treatment was performed the microbiological analysis of the samples. Microbiological studies were carried out by sowing on standard nutrient media, followed by incubation, counting the total microbial number, isolation of pure cultures and identification of microorganisms in accordance with the current methods ‘Rules for bacteriological research of feed,’ GOST R 51426-2016 ‘Microbiology. Feeds, complete feeds, feed raw materials. A general guide to preparing growing for microbiological research. ‘Type checking was confirmed by setting a ‘motley’ row and sowing pure culture on specific test systems (Nizhny Novgorod prod.).

As part of the study of the synergistic effect, when combining the effects of NEB and plasma radiation, were treated samples of ‘PK-5’ and ‘PK-6’ feed we. AD NEBs were in the range of 0-20 kGy, treatment of each sample with HP NGDP radiation was carried out for 3 minutes at a frequency of 316 Hz. Next, microbiological studies of samples were performed with determination of the total microbial number, isolation and identification of microorganisms. Physicochemical analysis was performed for control samples of feed, and samples subjected to maximum effects of NEB and HP GDP.

3. Results
Analysis of the microflora of control samples of feed showed a fairly high level of obscurity. Qualitative characteristics of samples are given in table 1. In tests of ‘PK-6’ were found the following strains of microorganism Candida albicans, Bacillus subtilis, Penicillium spp., in tests of ‘PK-5’ besides Candida albicans, Bacillus subtilis, Penicillium spp., found still Enterococcus faecium, Aspergillus spp. Higher values of microbial insemination of ‘PK-5’ samples (average contamination of 2.243 CFU/g samples) compared to ‘PK-6’ (1.157 CFU/g) are believed to be associated with longer shelf life of a given batch in a poultry plant.

![Figure 1](image-url)
Figure 1 shows the dependence of microbiological contamination of feeds on the amount of absorbed dose of NEB and treatment with HP GDP radiation. At AD $> 10$ kGy, microflora growth was absent. Pretreatment by HP GDP samples for 3 minutes reduces surface contamination, but the effect is selective.

Table 1. Microorganisms found in feed samples, NEB и HP GDP.

| Electrophysical influence | Found microorganisms                  |
|--------------------------|---------------------------------------|
| Plasma (min)             | NEB (kGy)                              | PK-5                           | PK-6                           |
| 0                        | 0                                     | Candida albicans, Bacillus subtilis, Enterococcus faecium, Aspergillus spp, Penicillium spp. | Candida albicans, Bacillus subtilis, Penicillium spp. |
| 0                        | 5                                     | Aspergillus spp., Penicillium spp. Candida albicans | Candida albicans, Penicillium spp. |
| 0                        | 10                                    | Candida albicans                | Candida albicans                |
| 0                        | 15                                    | No growth                       | No growth                       |
| 0                        | 20                                    | No growth                       | No growth                       |
| 3                        | 5                                     | Penicillium spp., Candida albicans | Penicillium spp., Candida albicans |
| 3                        | 10                                    | Penicillium spp.                | Candida albicans                |
| 3                        | 15                                    | No growth                       | No growth                       |
| 3                        | 20                                    | No growth                       | No growth                       |
| 3                        | 0                                     | Candida albicans, Bacillus subtilis, Aspergillus spp, Penicillium spp. | Candida albicans, Bacillus subtilis, Penicillium spp. |

4. Discussion and Conclusion

The ‘PK-5’ and ‘PK-6’ components included in the feed are a favorable nutrient medium for the development of bacteria, yeast and mold fungi, which, if conditions and shelf life are violated, leads to rapid deterioration of the feed. The use of microorganism-affected feed is not allowed, as it can cause diseases, poisoning of chickens and their death. Development of methods of feed’s disinfection will minimize the process of feed spoilage, increase storage life and increase their quality and safety.

As a result of studies, it was found that when exposed by NEB AD $= 5$ kGy, the average microbial insemination of the feed decreased by 19-25 times, while the proportion of sterile samples was more than 60%. Found in control samples of Bacillus subtilis, Enterococcus faecium, Aspergillus spp., in samples treated by NEB with AD $\geq 5$ kGy were not detected. Of the 40% samples showing signs of microbial growth after exposure by NEB were planted Aspergillus spp., Penicillium spp., Candida albicans. At AD $= 10$ kGy in some samples, only Candida albicans were detected in unit amounts.

The least resistant to NEB were the bacteria Bacillus subtilis, Enterococcus faecium, as well as representatives of the mold fungi Aspergillus spp. The most stable are yeast-like fungi of Candida albicans. At the same time, viable forms in feeds treated by NEB with a AD of $\geq 5$ kGy were detected only in a densely placed, knitted material. In samples where the feed was loose, the growth of microorganisms was absent, indicating a more uniform effect of NEB. Thus, by providing a more uniform treatment of the feed particles, for example by exposing the stream of loose material to NEB, the sterilizing dose can be reduced.
When studying the synergistic effect, it was found that, when exposed to feed samples ‘PK-5’ and ‘PK-6’ only by plasma radiation, without the use of NEB, the total microbial insemination decreased 2.5-4.5 times compared to the control samples but remained relatively high.

As mentioned above, the NEB treatment had a more pronounced bactericidal effect than the monotreatment of HP NGDP. In a combination of NEP and HP NGDP methods, it was found that the average values of the total microbial number of feeds were lower than in mono-treatment by NEB at the same doses. For example, at AD NEB 5 kGy, the mean of total microbial count was 113 CFU/g for ‘PK-5’ and 46 CFU/g for ‘PK-6’, and at the joint action of HP GDP and NEB, the total microbial count was 52 CFU/g and 6 CFU/g, respectively. From feed samples in which microorganism growth was observed after combined treatment, Candida albicans and Penicillium spp. were predominantly isolated.

The upper limit of the range of sterilizing doses with combined exposure to NEB and HP NGDP lay in the region of 12-15 kGy - the same as with monoprocessing by NEB (table 1). The lower limit was more dependent on the method of exposure: with the combination of HP NGDP and NEB with a dose of 5 kGy, the average microbial insemination of the feed decreased by 43 times for ‘PK-5’ and more than 380 times for ‘PK-6’ samples. At the same time, the proportion of sterile feed samples during monoprocessing by NEB 5 kGy was 60%, and with the combined effect of NEB in the same dose and HP GDP - 85% for ‘PK-5’ and 90% for ‘PK-6’. Thus, the synergistic effect of the combined treatment feeds by NEB and HP NGDP radiation is to shift the lower threshold of sterilizing doses downwards.

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