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Research Article

The Use of Nanosecond Electron Beam Irradiation Method to Sterilize Eggs in Industrial Poultry

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ABSTRACT:
The article describes a new method of bactericidal treatment for eating and hatchery eggs using a nanosecond electron beam irradiation with the accelerator of URT series. The dose sensitivity of the genus Salmonella pathogenic bacteria in vivo and the changes of microbial background concerning the surface of food eggs were shown during sterilization of a nanosecond electron beam irradiation (NEB) in a sealed package. Also the article demonstrates the results of researches concerning the influence of a nanosecond electron beam irradiation on the structure and a chemical compound of egg tissues. An analysis of egg incubation properties was performed. The eggs were irradiated by a nanosecond electron beam by an absorbed dose (AD) of 40 kGy. The article is devoted to the first results of electron-beam disinfection efficiency study concerning food and incubation egg surface .According to the obtained results, it is proposed to use the NEB sterilization method for eggs in industrial poultry farming.
Keywords: nanosecond electron beam, disinfection, sterilization, eating egg, hatchery egg, microbial content, Salmonella, radio sensitivity.

1. INTRODUCTION

The electro physical methods of food product and raw material processing are used successfully in certain branches of agricultural-industrial complex, which is conditioned by a number of advantages in comparison with more widespread, "traditional" methods [1]. When you sterilize with ionizing radiation, the temperature of a sterilized object rises slightly. In this regard such methods are called cold sterilization [2]. Due to this, it is possible to preserve the nutritional value of a product, avoiding the destruction of important protein molecules, vitamins, amino acids with a complete elimination of microflora from the product. Thus, the radiation sterilization of milk in radiotherapy doses recommended by the IAEA allows not only to reduce the microbial contamination to the limit of detection by laboratory methods, but also influences positively on certain quality indicators, such as raw state suitability, free amino acid content, etc. [1]. The equipment for the radiation sterilization must meet a number of requirements, represented by economic efficiency, the stability and reliability of parameters, the ease of maintenance and repair. The repetitive electrons accelerators of URT series meet these requirements to a large extent [3]. In order to study the method of egg disinfection with a nanosecond electron beam (NEB) irradiation, we selected the accelerators of this series, one advantage of which is the possibility of a product processing directly together with a package. The problem of an aseptic package and package sterilization is of great importance, since it is possible to

introduce microorganisms into an already sterilized product during this technological stage, which leads to its spoilage. The search for optimal product sterilization methods in a closed finished package is a promising trend of research [4,5]. However, the irradiation of the foods may be accompanied by a variety of chemical reactions which may transform the organoleptic properties of the products. Thus, it is necessary to set the limits of AD for irradiation of various products. For example, for fresh eggs, a level of AD ≤ 3 kGy is recommended, which is close to the AD level for inactivation of the bacteria of the Salmonella group (European, 2011). Irradiated foods are marked with a special sign "radura", so that the buyer could choose whether to use irradiated products or nor. Unfortunately, the radiation phobia is of great importance for the consumer choice. To solve the problems of microbiological contamination of eggs and the consumer sentiment, in our view, the following approaches look promising. Firstly, by proper electron energy selection, to choose such an AD distribution profile in the product depth, that will destroy, upon irradiation, all kinds of microbes, including the pathogenic ones both on the shell surface and in its pores as well as in the air chamber up to the under-shell membranes. In that way, there will be practically no exposure of the protein itself to the accelerated electrons. Secondly, the ozone will be produced under the irradiation, which will also contribute to the disinfection of the surface, especially by irradiation of the eggs sealed in plastic containers. It is possible, to sterilize the eggs after packaging by the irradiation itself as well as by the creation of ozone at a concentration levels lethal to microorganisms in the packaging – radiation-chemical sterilization (Kotov, 2000). At the same time, it is possible to select the AD distribution profile in the egg depth in such a way that, its albumen and yolk are not irradiated by electrons, at all. It is important that the presence of sealed plastic containers allows us to solve the problem of recontamination of eggs during storage. In order to evaluate the effectiveness of the method, we conducted a complex of studies for eating and hatchery eggs: we evaluated microbiological, physical-chemical, morphological, technological characteristics, we assessed the product compliance with the accepted quality standards and identified possible radiobiological effect.

A number of authors (Engohang-Ndong et al.) found that a substrate processing with a nanosecond electron beam with the energy of 3 MeV at an AD of 6.7 kGy is sufficient to reduce the total microbial contamination and the number of coliform bacteria to the standard values. The range of the radiobiological effect manifestation for enterobacteria made 2.7 - 30.7 kGy, the survival decreased with a dose increase, a complete elimination of microorganisms was noted at 30.7 kGy [8]. The study of spore form survival, conducted by S.E. Fiester and co-authors using the example of Bacillius, revealed a striking effect during the treatment of NEB in the range of 1.3 - 10.4 kGy [9]. In the course of the studies, we studied the ranges of dose sensitivity for three pathogenic strains of Salmonella genus bacteria in vitro; The sterilizing effect of NEB for a chicken egg surface was analyzed, the influence of NEB on the physicochemical properties of an egg was studied; The radiobiological effect was studied using the example of hatching eggs.

2. Characteristic of Radiation Sterilization Method for Chicken Eggs Based On Nanosecond Pulse of Accelerated Electrons

In order to process the eggs by the beam of accelerated electrons they used a pulse - periodic nanosecond accelerator URT-0.5 (electron energy up to 500 keV, the pulse duration of 50 ns, operation repetition rate up to 200 pps). On an infeed conveyor the plastic containers for 10 eggs are passed through the working space of the device, exposing to a uniform pulsed electron beam irradiation by width. After the processing the containers with eggs were inverted and the cycle was repeated.

During the processing of table eggs an absorbed dose were 5 and 25 kGy, during the processing of hatching eggs the corresponding dose was 40 kGy. The dosimetric control of an electron beam was carried out using film dosimeters at different points on a shell surface and under a shell, under a plastic container. In order to

determine the distribution of bremsstrahlung radiation (BR) absorbed dose in a chicken eggs they used thermal-luminescent dosimeters TLD-500. The dosimeters were placed in the sections of boiled eggs (cut lengthwise or crosswise) in order to make it possible to determine PD distribution in various points of an object. The measurement results showed that during the irradiation by an electron beam with PD of 5 kGy level sufficient for a full disinfection of a chicken egg surface, the absorbed dose within it will not exceed 8 cGy due to bremsstrahlung irradiation, which is not sufficient for the development of significant radiobiological effects in albumen and yolk.

3. Radiosensitivity of Salmonella Microorganism Study Using the Example of Sal. Enteritidis, Sal. Typhimurium, Sal. Gallinarum

In order to study the radio sensitivity of pathogenic microorganisms that can reside on the surface of eggs, they conducted the experiments with Salmonella microorganisms - the actual agents of food product microbial contamination. The representatives of Enterobacteriaceae family are facultative anaerobes which do not form spores. Enter bacteria include the representatives of the normal microflora among humans and animals, saprotrophs, as well as a number of opportunistic pathogens and pathogenic microorganisms, including the pathogens of serious infectious diseases among humans and animals. The most characteristic nutritional transmission mechanism of pathogenic enter bacteria is implemented through food and water, which conditions the crucial importance of food antimicrobial treatment to save their sanitary quality. We took the representatives of Salmonella three pathogenic serovars as a model organism: Salmonella enterica strain, serovar Typhimurium 79 (the strain № by passport in "GKPM-Obolenskaya": B-4376), serovar Gallinarum 665 (№ in "GKPM-Obolensk": B-4881) and serovar Enteritidis 11272 (the number in "GKPM-Obolenskaya": B-4846). The daily strain culture was prepared on a slant meat-peptone agar. Then the slurry of microorganisms was prepared with the concentration of 5 billion microbial cells in 1 ml according to turbidity standard and the seeding was performed by a surface method on elective nutrient medium Endo in petri dishes. Then the test samples were irradiated directly with NEB at the absorbed doses of 1.5 kGy, 5 kGy, 10 kGy, 25 kGy. The test samples were in the same conditions, but were not exposed to radiation treatment. Thereafter, the experimental and control samples were placed in an incubator and were incubated at 37±1 C for 16-18 hours. The consideration of results was performed visually by the number of colonies counting in the experimental and control samples. It was found that under the action on NEB microbial culture with the absorbed doses of 5 kGy, 10 kGy, 25 kGy microorganisms Salmonella typhimurium, Salmonella Gallinarum, Salmonella Enteritidis die completely, the growth of colonies on nutrient media is absent in test samples. At PD 1.5 kGy the growth of colonies was marked on the edge of the petri dish: 13.7 in Sal. typhimurium samples, 9,5 - in Sal. gallinarum samples, 8,8 in Sal. Enteritidis samples. The test ones had the growth in the range of 500 colonies. In order to confirm the results, they analyzed the biochemical properties of microorganisms (fermentation of sugars) and smear microscopy. It was established that the abovementioned properties in test samples correspond to the control ones, thus there was no introduction of foreign microorganisms in the course of the experiment.

Tab. 1. Survival of Sal. Typhimurium, Sal. Gallinarum, Sal. Enteritidis during the processing by a nanosecond electronic beam at different absorbed dose

№		CFU/g after NEB treatment*				
	Absorbed dose	***1,5 kGy***	***5 kGy***	***10 kGy***	***25 kGy***	***0 kGy - control***
1	Salmonella typhimurium	13,7	No growth	No growth	No growth	~500
2	Salmonella Gallinarum	9,5	No growth	No growth	No growth	
3	Salmonella Enteritidis	8,8	No growth	No growth	No growth	

*The amount of Colony Forming Units (CFU), survived after NEB treatment with applied to the nutrient media of a standard suspension (1 billion of microbial cells per 1 ml)

4. Estimation of Neb Disinfecting Action Efficiency on Chicken Egg

The bactericidal effect of a nanosecond electron beam on the egg microflora was evaluated using the example of table eggs and hatchery eggs. Swabs were taken for bacteriological and bacterioscopic analysis from the test eggs of the control and experimental batches, then an experimental batch of table eggs in plastic packaging containers was subjected to an experimental irradiation by a nanosecond electron beam. The control batch (n = 180) was in the same conditions as the experimental one, but it was not subjected to NEB action. In experimental batches, the absorbed dose was 5 kGy (n = 240) and 25 kGy (n = 240). Immediately after the irradiation, swabs were taken from the egg surface and subjected to microbiological examination - sowing was carried out on nutrient media with the isolation of cultures and the identification of the obtained microorganisms. Eggs were stored for 25 days at the temperature of 0-20 C and the humidity of 85-88%. On the 12th and 25th day, the second and the third microbiological control of the experimental and control batches was carried out: the washing off from the surface of eggs, followed by the sowing on nutrient media, the isolation and identification of microorganisms. The result of the conducted studies showed that the processing of table eggs in commercial plastic package with a nanosecond electron beam suppresses the growth of microflora on the surface of eggs completely with an absorbed dose of 5 kGy and more. If the storage conditions are observed, the surface of the eggs remains a sterile one throughout the whole regulated shelf life. The growth of St. aureus was noted in control samples.

Tab.2. Microbiological evaluation of table egg processing efficiency by NEB method

Processing method	Control, CFU/g(cm^3)			Experience		
	1st day	12th day	25th day	1st day	12th day	25th day
NEB, absorbed dose 5 kGy	St.aureus - 400	St.aureus - 2900	St. aureus - 16000	No growth	No growth	No growth
NEB, absorbed dose 25 kGy	St.aureus - 600	St.aureus - 660	St. aureus - 16000	No growth	No growth	No growth

The microbiological study of the hatchery eggs was carried out during the first day: prior to the impact by the beam of accelerated electrons and after it, just before its laying in an incubator. The control batch (n = 50) was under the same conditions as the experimental one, but it was not exposed to NEB. The absorbed dose during egg processing (n = 100) of NEB was 40 kGy, which is 4 times higher than the maximum dose for food product radiation sterilization recommended by IAEA. The choice of a high absorbed dose is dictated by the need to evaluate the damaging effect of radiation on egg tissues. Microbiological analysis of swabs from the egg surface of the experimental batch revealed a complete absence of microorganism growth.

5. The Study of Neb Effect on Physical-Chemical Composition and Structure of Chicken Eggs

In order to analyze the mass fraction of vitamin A in the yolk and vitamin B2 in the albumen, a high-performance liquid chromatography (HPLC) method was used. It was found that the content of vitamin A in the egg yolk subjected to nanosecond pulses of accelerated electrons is slightly higher than that for the eggs of the control batch: at an absorbed dose of 5 kGy 9450 ± 372.2 IU (2.83 ± 0.10 mg/kg), at an absorbed dose of 25 kGy 8683 ± 670.2 IU (2.59 ± 0.11 mg/kg). In the control samples, the vitamin A content in the yolk was 7647 ± 310.20 IU (2.79 ± 0.10 mg/kg). The content of vitamin B2 in the albumen of eating eggs was 7.06 ± 0.31 mg/kg in the control batch, 3.55 ± 0.42 mg/kg in the experimental batch (NEB, 5 kGy), and 2,24 ± 0.12 mg/kg in the experimental batch (NEB, 25 kGy). The mass fraction of the shell calcium in the experimental and control samples did not have statistically significant differences and made on the average 366112.4 ± 18310.20 mg/kg (test samples) and 368192.7 ± 18399.61 mg/kg (control samples). The content

of sodium in the shell was different, and was less in the control samples (908.5 mg/kg) than in the experimental ones under the action of accelerated electrons: 1324.2 mg/kg (PD 5 kGy) and 2064.5 mg/kg (PD 25 kGy). The content of phosphorus in a shell did not represent a reliable difference between all experimental and control samples.

Tab.3. Vitamin content in protein

Analysis type	The content of vitamins in IU (mg/kg) at absorbed dose			Control (absorbed dose – 0 g) in IU (mg/kg)	
	5 kGy	25 kGy	40 kGy	Edible egg	Hatched egg
Vitamin A in yolk	9450±372 (2,83±0,10)	8683±670 (2,59±0,11)	13370±559 (3,41±0,17)	7647±310 (2,79±0,10)	13731±622,70 (4,12±0,17)
Vitamin B_2 in protein	(3,55±0,42)	(2,24±0,12)	-	(7,06±0,31)	-

Tab.4. Content of elements in a shell

Element	The content in mg/kg at absorbed dose		**Control (0 g), mg/kg**
	5 kGy	**25** kGy	
Calcium	366112,4±18310,2		368192,7± 18399,6
Sodium	1324,2	2064,5	908,5

The analysis of albumen substance content in eggs did not reveal statistically significant changes and differences between the samples of all experimental and control groups. During the study of amino acids, it was found that the content of lysine undergoes greatest changes: the smallest amount was noted in control samples (0.88% ± 0.05%). The highest values were recorded in the samples subjected to the action of NEB (absorbed dose was 25 kGy) - 1.15% ± 0.05%. The changes in the percentage content of tryptophan and methionine were insignificant among all groups. The internal structure of the hatchery eggs was examined by the methods of ovoscopy and visual evaluation. There noted a tendency to the number of dots, rods and large pores increase on shell, as well as the appearance of foreign inclusions in albumen and yolk during the 25th day as compared with similar indicators during the first day of the experiment. All the revealed changes of shell, albumen, yolk and air cell qualities and properties corresponded to the natural changes in an egg structure during its long storage and they were relatively similar in the experimental and control groups. There were no statistically significant differences between the groups according to these characteristics.

6. Evaluation of Neb Radiobiological Impact on Hatching Egg Embryo

The damaging effect of ionizing radiation is caused both by direct action on biomolecules and, indirectly, through the formation of oxygen active forms, which oxidize biomolecules. And this leads to the disruption of a cell vital activity [10]. The most radiosensitive cells are the cells of rapidly regenerating tissues, as well as the cells of embryos and fetuses. In order to study the damaging radiation effect of a nanosecond electron beam on the hatchery egg tissues and a developing embryo, ovoscopy and macroscopic analysis of the quality of the egg were performed, and incubation was carried out according to the standard technology used in industrial poultry farming. The percentage of produced eggs and the quality of the young was assessed daily during the growing period before the slaughtering (37 days). They studied biochemical, immunological and hematological indices of broiler chicken blood to identify possible metabolic and physiological disturbances. A pathological anatomical study was conducted after the slaughtering of the experimental and control group chickens. A macroscopic analysis of the hatchery egg quality showed that the parameters of the albumen, yolk and eggshell from the control and test groups are very similar in value: thus, the average egg albumen diameter of the experimental and control groups was within 79.2 - 80.8 mm, the height was 7,6 mm, the albumen index was 0.1%, the yolk diameter varied in the range of 43.5-44.5 mm, the yolk index was 0.4%. The shell weight made 7.4 g, the thickness of the shell was 0.32 mm, the elastic deformation was 22.5 μm in the control batch, and similar parameters were observed in the experimental batch - 7.5 g, 0.33 mm and 22.0 μm, respectively. The ovoscopic assessment of eggs revealed insignificant differences in a shell

structure: eggs with a large number of dots and rods on shell were larger in the experimental group (80% of the total number) than in the control group (53%). The remaining parameters - the structure of albumen, yolk, air chamber, the presence of pathological inclusions did not have a statistically significant difference. After egg incubation, they calculated the percentage of withdrawal which was 63% in the control group and 64% in the experimental group. A path morphological study of unborn chickens and the ones who died the first hours showed that the reason for the chicken fading and death were the natural inaccuracies of incubation. There were no morphological signs of radiation damage and its consequences among embryos.

Tab.5. Results of hatched egg quality microscopic analysis

Parameter	Control	Test (40 kGy)
Average diameter of protein, mm	79,2 - 80,8	
Height of egg protein, mm	7,6	
Protein index, %	1	
Yolk diameter, mm	43,5-44,5	
Yolk index, %	0,4	
Shell weight, g	7,5	7,4
Shell thickness, mm	0,33	0,32
Elastic deformation, μm	22	22,5
Number of dots and rods on shell, %	53	80
Hatch, %	63	64

7. CONCLUSIONS

On the basis of the stated above, it can be concluded that the use of a nanosecond electron beam for the disinfection of chicken eggs allow to eliminate the pathogenic microflora on the surface and in the pores of shell effectively without affecting the internal structures of an egg. The dose of 5 kGy is the sterilizing dose sufficient to kill the microorganisms on the surface of eggs in a plastic package. When you process eggs, at a dose exceeding the minimum sterilizing one in 8 times (40 kGy), no significant changes in the morphological physical-chemical properties of albumen, yolk, air cell and shell were detected. The radiobiological effect is not expressed, since the hatchability and chicken health status indices obtained from the NEB treated eggs during the first hours of embryo development did not differ from the corresponding control group indicators.

8. CONCLUSION

Thus, the use of the nanosecond electron beam method for the disinfection of food and hatching eggs seems to be promising one for industrial poultry farming, since it provides a pronounced bactericidal effect maintaining the food and incubatory qualities of an egg. The advantage of this method is the possibility of already packed product processing, which eliminates the repeated contamination of eggs with microorganisms. It is also possible to use NEB for the disinfection of containers, inventory and other objects of poultry farming.

9. CONFLICT OF INTERESTS

The authors confirm that the presented data do not contain any conflict of interest.

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